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THE MINERAL METABOLISM OF THE BEEF STEER

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THIS study was undertaken, in connection with an investigation of The Energy Metabolism of Cattle in Relation to the Plane of Nutrition (1), for the purpose of establishing a basis for the comparison of the mineral metabolism of the beef steer with that of the milch cow, the latter having become fairly well understood through recent extensive research, while the former has not been made the subject of similarly thoroughgoing studies.

A series of 60 complete mineral balances with milch cows, by the senior author and former associates (2), has revealed the mineral metabolism of the cow to be characterized in part as follows: The selective improvement of this species for milk production has so increased the feed requirement that this cannot be satisfied by roughage alone (because of its bulk) but must be met in large part by concentrates, which are, on the whole, comparatively poor in calcium and in vitamin D; has increased the calcium and phosphorus requirements to quantities which, under many conditions, apparently exceed the cow's capacities of assimilation; and has prolonged the period of lactation, and has timed the same, so that the cow is called upon to produce milk in abundance from dry feed during the winter time, when the vitamin D of the available feeds and the sunshine, both of which are influential in the control of the metabolism of calcium and phosphorus, are less in evidence than during the summer, the season of abundant milk secretion in the cow in a state of nature.

There has come about, therefore, a more or less critical situation with regard to the mineral metabolism of the milch cow, in which negative balances of calcium at least usually, and of phosphorus almost as commonly, prevail during the early part of the period of lactation in the

nutrition of the ordinarily well-fed animal, especially in the winter time, and regardless of geographic location.

Later, when the milk production and the consequent demands for mineral nutriment have decreased, positive balances of calcium and of phosphorus come to prevail, and there is repayment of the earlier mineral overdrafts from the skeleton, the rate and completeness of which is influenced by the composition of the ration, especially of the forage, as determined by its botanical character—whether leguminous or gramineous—and by the method by which the dry forage has been cured.

The significant unit of time in this physiological and nutritional problem is the annual cycle of lactation and gestation, and the final effect of methods of feeding and other treatment on the annual mineral balance of the cow has not been determined in detail and with definiteness; but throughout the whole of this situation, and during the prevalence of negative balances of calcium and of phosphorus, the ordinarily well-fed milch cow is peculiarly unresponsive to increases in the calcium and phosphorus contents of the ration brought about by the feeding of mineral supplements.

The negative calcium and phosphorus balances in the metabolism of the ordinarily well-fed milch cow, therefore, are not caused by deficient intake of these elements,—in the light of which fact it is not logical to attempt to prevent these negative balances by the feeding of mineral supplements.

In addition to this problem, which is primarily physiological, there is a local problem, affecting cattle of all kinds, which results from abnormal characteristics of the feed, especially the forage—usually due to peculiarities of composition of the soil; and in this general situation cattle suffering from deficient mineral intake are normally responsive to the feeding of mineral supplements, and may be very greatly improved in general physical condition by this means.

For comparative purposes in the normal, physiological and nutritional limitation of the milch cow, and also in the local, mineral starvation of cattle in general, as a result of peculiarities of feed composition, it would be helpful to know the character of the mineral metabolism of the growing and fattening steer, on normal feeds and at various levels or planes of nutrition. In this light the following account of experiments is presented.

A duplicate series of balances of calcium, magnesium, phosphorus, and nitrogen, was determined with two Aberdeen-Angus steers, designated Nos. 47 and 36, and aged 25 to 31, and 27 to 33 months, respectively.

As will be observed in Table I, there were five balance experiments with each steer, eight of these ten periods being 18 days each in length, and the other two twelve days and thirteen days, respectively.

TABLE I. SCHEDULE OF EXPERIMENTATION

Period No.	Steer No.	Days in experimental period	Preliminary feeding periods on experimental rations	Experimental periods	Planes of nutrition	Live weight of animals
						Kilos
1	47	18	Nov. 24–29	Nov. 30–Dec. 17	Twice maintenance	486.2
2	36	18	Dec. 8–13	Dec. 14–31	do	482.9
3	47	18	Dec. 21–27	Dec. 28–Jan. 14	Half more than maintenance	494.6
4	36	18	Jan. 1–10	Jan. 11–28	do	490.2
5	47	12	Jan. 14–24	Jan. 25–Feb. 5	Half maintenance	474.8
6	36	13	Jan. 29–Feb. 6	Feb. 7–19	do	471.2
7	47	18	Feb. 5–14	Feb. 15–Mar. 4	Maintenance (mixed ration)	484.8
8	36	18	Feb. 21–28	Mar. 1–18	do	481.2
9	47	18	Mar. 11–14	Mar. 15–April 1	Maintenance (hay only)	499.0
10	36	18	Mar. 23–28	Mar. 29–April 15	do	499.9

Each balance period was preceded by a preliminary feeding interval, not less than 10 days in length, during which the steers received the same kinds of feed that were to be given during the experimental period to follow; while the preliminary intervals during which the steers received exactly the same quantities of feed as during the experimental periods were not less than five days in length, as indicated in the first column of dates.

The rations in eight of the ten experimental periods were composed of corn (maize) meal and alfalfa hay, in equal weights, and in the remaining two periods were alfalfa hay alone; and the planes of nutrition were such as to supply—of energy-producing nutriment—(1) twice the maintenance* requirement, (2) half more than maintenance, (3) half of the maintenance requirement, (4) maintenance (hay and grain, as in previous treatments), and (5) maintenance (alfalfa hay alone).

* Throughout this paper the word, "maintenance," unless otherwise qualified, signifies energy-equilibrium.

Sodium chloride was fed in the amount of 30 grams per head, per day, at all times.

The rations, therefore, were composed of normal feeds, and were not known to be characterized by any nutritive deficiency, except as the plane of nutrition was below the maintenance level.

The daily quantities of calcium, magnesium, phosphorus, and nitrogen, in the feed and excreta, and the balances of these elements, in the experimental periods, at the four planes of nutrition, for the two steers, are given in Table II.

TABLE II. AVERAGE DAILY BALANCES OF CALCIUM, MAGNESIUM, PHOSPHORUS, AND NITROGEN. Steer No. 47

Period number	Plane of nutrition	Average daily ration	Calcium Feed Feces Urine Balance	Magnesium Feed Feces Urine Balance	Phosphorus Feed Feces Urine Balance	Nitrogen Feed Feces Urine Balance
		Grams	Grams	Grams	Grams	Grams
1	Twice maintenance	Corn meal, 4290 Alfalfa hay, 4290	35.103 31.559 0.127 +3.417	16.272 10.780 2.383 +3.109	22.437 13.991 2.793 +5.653	159.8 58.1 87.1 +14.6
3	Half more than maintenance	Corn meal, 3250 Alfalfa hay, 3250	26.666 23.499 0.056 +3.111	12.327 9.374 2.136 +0.817	16.997 8.883 4.151 +3.963	121.5 40.6 74.2 + 6.7
7	Maintenance	Corn meal, 2188 Alfalfa hay, 2145	17.523 17.103 0.057 +0.363	8.419 6.122 1.767 +0.530	10.830 5.660 6.220 −1.050	79.4 23.7 47.9 + 7.8
5	Half maintenance	Corn meal, 1083 Alfalfa hay, 1073	8.769 8.317 0.074 +0.378	4.200 2.253 1.087 +0.860	5.389 3.415 2.459 −0.485	39.0 11.7 41.3 −14.0
9	Maintenance (hay only)	Alfalfa hay, 6500	52.470 50.696 0.121 +1.653	16.111 14.640 1.613 −0.142	16.640 7.976 4.402 +4.262	145.8 47.6 89.5 + 8.7

TABLE II (Continued). AVERAGE DAILY BALANCES OF CALCIUM, MAGNESIUM, PHOSPHORUS, AND NITROGEN.

Steer No. 36

Period number	Plane of nutrition	Average daily ration	Calcium Feed Feces Urine Balance	Magnesium Feed Feces Urine Balance	Phosphorus Feed Feces Urine Balance	Nitrogen Feed Feces Urine Balance
		Grams	Grams	Grams	Grams	Grams
2	Twice maintenance	Corn meal, 4088 Alfalfa hay, 4048	33.007 28.666 0.083 +4.258	15.309 10.882 3.582 +0.845	21.108 16.833 0.679 +3.596	152.2 57.6 83.4 +11.2
4	Half more than maintenance	Corn meal, 3090 Alfalfa hay, 3090	25.302 23.060 0.060 +2.182	11.748 8.284 2.321 +1.143	16.161 11.654 1.513 +2.994	115.9 39.5 70.9 + 5.5
8	Maintenance	Corn meal, 2181 Alfalfa hay, 2155	17.599 17.370 0.047 +0.182	8.473 5.715 1.742 +1.016	10.838 7.326 2.535 +0.977	78.8 23.0 47.1 + 8.7
6	Half maintenance	Corn meal, 1088 Alfalfa hay, 1073	8.774 8.958 0.107 −0.291	4.208 2.909 0.952 +0.347	5.402 3.260 2.961 −0.819	39.5 11.4 41.9 −13.8
10	Maintenance (hay only)	Alfalfa hay, 6500	52.444 49.264 0.108 +3.072	16.089 13.981 1.429 +0.679	16.640 10.480 2.416 +3.744	145.6 48.2 89.0 +8.4

In the choice of the above mentioned elements for consideration in this study calcium and phosphorus were included as of the greatest quantitative significance, and also as most likely to be deficient in the rations of cattle, while magnesium was included because of its association with calcium and phosphorus in the skeleton and in metabolism generally, and nitrogen as an indication of the status of the general metabolism of active tissue.

During the maintenance periods on the ration of corn meal and alfalfa hay (periods 7 and 8) the balances of calcium, and magnesium, were all

positive; that is, there was retention, or storage, of these elements in the body, the positive balance of calcium being small, but significant, while the retention of magnesium was more extensive.

In the case of phosphorus there was with one steer a small positive balance and with the other about an equal loss of this element.

With both steers there was a significant gain of nitrogen.

An energy-maintenance ration of corn meal and alfalfa hay, in equal parts, therefore, supplies enough calcium, magnesium, phosphorus, and nitrogen to maintain equilibrium of these elements in a two-year-old steer.

Above the plane of maintenance the retention of calcium and phosphorus was in each case increased in consistent relation with increased intake of the same; and the retention of magnesium also generally increased with increased intake of this element; but in one case, with steer No. 36, the magnesium retention at the highest plane of nutrition seems to have been a little less than at the two lower planes of nutrition. While this seems inconsistent, in a sense, it is in harmony with certain facts observed by Forbes and associates in their studies of the mineral metabolism of milch cows and of swine.

Thus, impressively large balances—either positive or negative in character—of mineral elements contained in the ration in superabundant quantities, but used in the body in relatively small quantities, are frequently encountered and may be regarded as unimportant fluctuations in extensive reserves, in response to undetermined influences,—these balances, therefore, being without significance as indications of the sufficiency or the insufficiency of the intake. It is, therefore, impossible, sometimes, to say what a particular mineral balance signifies. This applies especially to balances of magnesium and potassium. There is much less ground for question, in this regard, as to the significance of balances of elements such as calcium and phosphorus which, during growth and lactation, may be utilized in considerable proportions of the quantities consumed. Balances of calcium and phosphorus, therefore, mean much more nearly what their face-value signifies than do those of magnesium and potassium, which, at face value would often be most misleading.

On a maintenance ration of alfalfa hay alone (periods 9 and 10) the intake of calcium was three times as great, and of phosphorus about one-and-a-half times as great, as on the maintenance ration of corn and alfalfa hay (periods 7 and 8).

A comparison of the calcium and phosphorus balances from the maintenance rations of corn and alfalfa hay with similar balances from the

maintenance rations of alfalfa hay alone indicates that the increased content of phosphorus in the latter was much more efficiently utilized than was the increase in calcium; and with both steers the corresponding increase in magnesium led to a decrease in magnesium retention.

These data exemplify the fact that the balances of the mineral nutrients are affected by such a complication of influences,—solubility of compounds present, interaction of inorganic acids and bases in solution in the alimentary tract and in the blood, absorption from the alimentary tract, relation to carbohydrate and fat metabolism, utilization by synthesis in body tissues (especially as limited by the quantities of associated nutrients present), fluctuations in the nutrient reserves of the body, and elimination by kidneys and intestine—that it is impossible to interpret closely, and with assurance, the differences in mineral balances resulting from differences in amounts and kinds of feeds consumed. Information of a critical and fundamental character, as to mineral metabolism, can be derived only from very long continued or very specially planned investigations.

At the level of half of the maintenance requirement the nitrogen balances were consistently negative, as also were the balances of phosphorus. The magnesium intake seemed to be sufficient; while the balances of calcium were divided as to character, one positive and one negative. Such apparent inconsistencies as the disagreement in the sign of the calcium balances might readily be due to individual differences in the metabolism of fat and of calcium soaps formed in the intestine.

Mineral balances representing only a few days, or a few weeks, therefore, must be considered to be of questionable and of differing significance (according to the element in question), and, in order to be of positive and definite value, mineral balances with growing steers should represent a considerable part, at least, of the life cycle—at least several months—and, as has been observed, the significant unit of time in the mineral metabolism of the cow is the annual cycle of lactation and gestation.

Summary of Conclusions

A series of 10 nutritive balances of the calcium, magnesium, phosphorus, and nitrogen of normal feeds at four planes of nutrition, with each of two two-year-old steers, was determined in experimental periods mostly 18 days in length.

The planes of nutrition studied were, (a) half of the energy-maintenance requirement, (b) maintenance, or energy equilibrium, (c) half more than maintenance, and (d) twice maintenance.

A ration of corn (maize) meal and alfalfa hay, in equal weights, was fed at each of the four planes of nutrition; and in addition a ration of alfalfa hay alone was fed at the energy-maintenance level, with each steer.

The mineral metabolism of the steers was found to be of the normal character for growing animals, and not to be characterized by the peculiarities of the mineral metabolism of the milk-producing cow.

The balance data signify that with the mixed ration of corn meal and alfalfa hay, at planes of twice maintenance, half more than maintenance, and maintenance, the intake of calcium, magnesium, phosphorus, and nitrogen was adequate; that at the plane of half of the energy-maintenance requirement the intake of magnesium was adequate, of calcium was at about the point of equilibrium, and of phosphorus and nitrogen was definitely insufficient; while with the ration of alfalfa hay alone at the maintenance level the intake of all elements studied was adequate.

Eighteen-day balances of mineral elements such as calcium and phosphorus, which are utilized to the extent of considerable proportions of the usual intake of the same, are of value as indicating the adequacy of the quantities present to serve the nutritive requirements of the animal, though a continuous series of such balances would yield more reliable data; but balances of mineral nutrients such as magnesium, which are utilized in small proportions of the usual intake, are of much less certain significance, and cannot be closely interpreted—especially in view of the great complication of factors, other than the quantity present, which enter into the determination of the balance of intake to outgo.

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THE IRON CONTENT OF THE ALBINO RAT AT DIFFERENT STAGES OF THE LIFE CYCLE*

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IN THIS study determinations were made of the normal iron content of the bodies of albino rats, at intervals throughout the life cycle, especially for the purpose of establishing a basis for the interpretation of the studies reported by Miller, Forbes and Smythe (1) in the following paper.

Some early observations bearing on the subject of this study are recorded in the literature. Bunge (2) first observed that the percentage of iron in the ash of new-born animals is much higher than the percentage of iron in the milk on which they are nourished. This was found true of the dog, the cat and the rabbit, the percentage in the body in some cases being six times that in the milk. It was also shown that the percentage of iron in the body gradually falls during the suckling period, and reaches its lowest point just before the animal begins to eat solid food. These observations, however, were not found to be true of the guinea pig, probably because of the fact that this animal eats solid food at a very early age. Work of Abderhalden (3) supports these findings of Bunge.

Certain observations of the hemoglobin content of animals also have a bearing on the subject of this study. Thus Abderhalden (4), (5), Chisholm (6), and Williamson and Ets (7) have reported the hemoglobin content of the rat at various ages. While their results are not in strict agreement, they are all of similar significance in showing that the percentage of hemoglobin gradually diminishes during the suckling period. Nicholas (8) has studied the hemoglobin content of rat fetuses, and finds that the storage of hemoglobin is most rapid during the early part of the last third of the gestation period. This is in agreement with the finding of Hugounenq (9) that, in the case of the human fetus, the storage of iron takes place mainly during the last three months before birth.

EXPERIMENTAL

The methods of experimentation and analysis employed in this study were the same as in the following paper (1). The main part of the diet of

* The data on which this paper is based were taken from a thesis presented by C. V. Smythe to the Graduate Faculty of the Pennsylvania State College as a part of the requirements for the degree of master of science.

the rats consisted of a commercial calf meal of the following composition:

Yellow corn meal	25 parts
Wheat red-dog flour	22 "
Oat flour	15 "
Linseed oil meal	15 "
Blood flour	10 "
Ground malted barley	10 "
Sodium chloride	1 "
Precipitated calcium carbonate	1 "
Precipitated bone	1 "

To this were added five parts of cod liver oil and five parts of wheat germ middlings, the entire mixture containing 22 per cent of crude protein and 0.027 per cent of iron.

The stock from which a part of the rats used in this study were derived had been fed this diet during the past ten generations. It therefore appears to be adequate for complete nutrition.

Iron was determined in the bodies of the rats at birth, and at the ages of 9, 20, 40, 60 and 100 days; also a number of adults were analyzed, including females at the time of parturition, at the time of weaning their young, and at thirty days after the weaning.

Results and Discussion

The iron contents of the bodies of the rats of the seven age groups, from birth to maturity, are summarized in Table I, and a graphic representation of these data is presented in Figure I. The individual results are given in Tables II to VIII; while in Table IX are given the iron contents of female rats in various conditions with reference to the reproductive function.

Table I. Iron Content of Rats at Various Ages

Age of rats	No. of rats analyzed	Average body weight	Average iron in body	
		gms.	mgs.	per cent
at birth	18	5.0539	0.279	0.0055
9 days	7	10.4668	0.398	0.0038
20 days	6	32.3035	0.806	0.0026
40 days	9	47.5680	2.221	0.0045
60 days	10	98.7369	4.416	0.0045
100 days	7	134.4446	5.835	0.0043
adult	6	211.8532	10.780	0.0051

Figure 1 shows that the percentage iron content of rats decreases very rapidly during the suckling period, after which it rises rapidly until the rats are about 40 days old. After this age the iron content remains almost constant, and is practically the same as at birth. Therefore the rat can

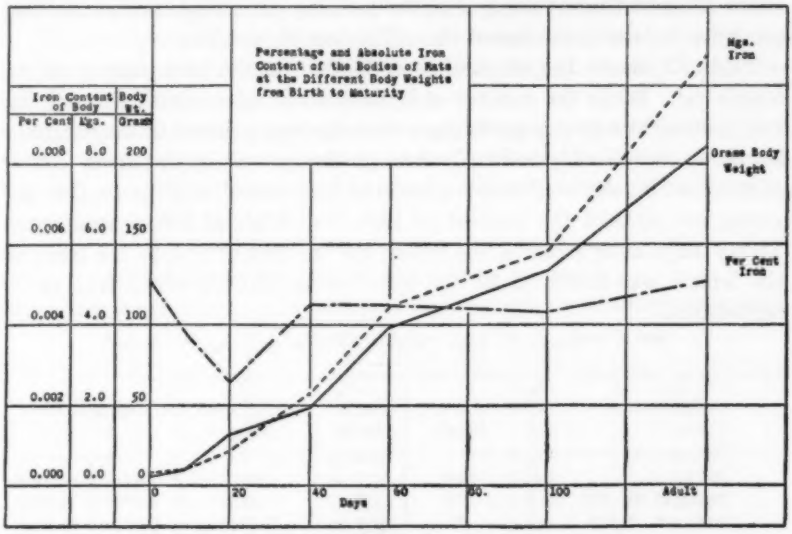

FIGURE 1

hardly be considered as having a reserve supply of iron in its body at birth. Rather, it is born with a percentage iron content about the same as that of the adult, and during the suckling period reaches a condition which may be designated as normal physiological milk anemia.

Slight differences in iron content, apparently due to the number of young in a litter, to the age and breeding history of the mother, and to unexplained individuality were also encountered.

The curve representing the absolute amounts of iron found in the rats corresponds rather closely to the growth curve. Attention is called to the fact that in spite of the marked decrease in the percentage of iron in the bodies of the rats during the suckling period, there was during this time an appreciable absolute storage of iron.

These observations signify that weanling rats, at ages of 24 to 28 days, such as were used by Miller, Forbes and Smythe (1), can at the most have barely had time, since being weaned, to bring the percentage iron content of their bodies to the normal, and were probably in a state of moderate, physiological milk anemia.

Waddell and associates (10) discussed the difficulty experienced by certain workers in producing anemia in rats, and cited instances of the use of animals from the second generation on deficient diets as a means of obtaining anemic subjects. The curve of iron percentages in Figure 1 seems to show that at the end of the suckling period rats are in an ideal condition for use in studies of the utilization of food iron.

Table IX shows the effect of reproduction on the iron content of the female rat. While the number of individuals involved in this consideration is small the data seem to show that the iron content of the pregnant female is considerably reduced, through the growth of the litter. After parturition the depleted reserves begin to be restored, and by the time the young are weaned the content of iron is as high as before pregnancy. Thirty days after weaning the young the content of iron in the body of the female was found to be the same as in controls which had never reproduced.

TABLE II. IRON CONTENT OF RATS AT BIRTH

Rat number	Age of female	No. in litter	Weight of body	Iron in body	
	days		gms.	mgs.	per cent
Young of No. 103	130	7	4.6338	0.194	0.0042
" " " "	"	"	5.0933	0.222	0.0044
" " " "	"	"	4.0608	0.200	0.0049
" " " "	"	"	4.3880	0.231	0.0053
" " " "	"	"	4.3155	0.200	0.0046
" " " 71	180	6	5.0595	0.333	0.0066
" " " "	"	"	4.2717	0.286	0.0067
" " " "	"	"	4.7810	0.294	0.0062
" " " "	"	"	5.3725	0.315	0.0059
" " " "	"	"	4.7146	0.283	0.0060
" " " "	"	"	3.9605	0.222	0.0056
" " " 404	150	4	6.5258	0.424	0.0065
" " " "	"	"	5.8102	0.339	0.0058
" " " "	"	"	5.8950	0.362	0.0061
" " " 157	120	8	5.1353	0.256	0.0050
" " " "	"	"	5.2777	0.265	0.0050
" " " 135	120	6	6.0134	0.317	0.0053
" " " "	"	"	5.6624	0.283	0.0050
Average			5.0539	0.279	0.0055

SUMMARY

The normal iron contents of the bodies of albino rats at different ages, and in different conditions as to reproductive activity, are reported.

The average iron content of rats at birth was found to be 0.0055 per cent. A decrease to 0.0026 per cent occurred during the suckling period. A pronounced increase, to 0.0045 per cent, took place between the ages of 20 to 40 days, after which only slight variations occurred. This increase in iron content apparently resulted from the taking of solid food.

The absolute amount of iron present increased very slowly during the suckling period, but more rapidly thereafter, and at a rate closely proportional to the increase in body weight.

The iron content of females was depleted through the growth of the litter during pregnancy; but the analysis of a single individual after the weaning of the young indicates that the iron content of the female returned to the normal during lactation.

TABLE III. IRON CONTENT OF 9 DAY OLD RATS

Rat number	Weight of body	Iron in body	
	gms.	mgs.	per cent
Young of No. 84	9.3263	0.299	0.0032
" " " "	9.3959	0.255	0.0027
" " " 73	10.0637	0.470	0.0047
" " " "	9.7745	0.387	0.0040
" " " "	7.9538	0.405	0.0051
" " " 81	13.9526	0.497	0.0036
" " " "	12.8006	0.472	0.0037
Average	10.4668	0.398	0.0038

TABLE IV. IRON CONTENT OF 20 DAY OLD RATS

Rat number	Sex	Weight of body minus alimentary tract	Iron in body	
		gms.	mgs.	per cent
Young of No. 73	Female	24.3368	0.769	0.0032
" " " "	Male	24.6390	0.738	0.0030
" " " "	Male	22.4354	0.667	0.0030
" " " 90	Female	39.9900	0.840	0.0021
" " " "	Male	40.7740	0.885	0.0022
" " " "	Male	41.6460	0.935	0.0022
Average		32.3035	0.806	0.0026

TABLE V. IRON CONTENT OF 40 DAY OLD RATS

Rat number	Sex	Weight of body minus alimentary tract	Iron in body	
		gms.	mgs.	per cent
84	Male	49.7170	2.475	0.0050
85	Male	56.9100	2.741	0.0048
86	Male	66.7130	3.291	0.0049
87	Male	54.7150	2.873	0.0053
88	Male	69.2330	3.486	0.0050
264	Male	28.1161	0.913	0.0032
265	Female	32.0200	1.136	0.0035
266	Female	26.6876	1.198	0.0045
306	Male	44.0000	1.875	0.0043
Average		47.5680	2.221	0.0045

TABLE VI. IRON CONTENT OF 60 DAY OLD RATS

Rat number	Sex	Weight of body minus alimentary tract	Iron in body	
		gms.	mgs.	per cent
290	Male	75.6062	3.189	0.0042
294	Male	79.8233	3.221	0.0040
298	Male	62.0779	2.658	0.0043
325	Male	114.5583	5.207	0.0045
329	Male	117.3696	5.621	0.0048
333	Female	108.9186	4.607	0.0042
337	Male	117.3906	5.243	0.0045
341	Female	100.0365	4.956	0.0050
345	Female	88.1580	4.025	0.0046
429	Male	123.4303	5.433	0.0044
Average		98.7369	4.416	0.0045

TABLE VII. IRON CONTENT OF 90–120 DAY OLD RATS

Rat number	Age	Sex	Weight of body minus alimentary tract	Iron in body	
	days		gms.	mgs.	per cent
33	92	Male	119.1544	5.297	0.0044
37	92	Male	112.7160	4.970	0.0044
41	92	Male	111.5610	4.764	0.0043
45	92	Male	88.8700	3.857	0.0043
471	90	Male	199.9205	9.919	0.0050
67	120	Male	154.2705	6.102	0.0040
71	120	Male	154.6198	5.934	0.0038
Average			134.4446	5.835	0.0043

TABLE VIII. THE IRON CONTENT OF ADULT RATS

Rat number	Sex	Age	History	Weight of body minus alimentary tract	Iron in body	
				gms.	mgs.	per cent
358	Female	15 mo.	Would not breed after having one litter	198.5490	8.633	0.0043
385	Female	12 mo.	Had one litter	163.0279	9.383	0.0058
389	Female	Unknown	Unknown	170.4396	8.954	0.0053
417	Male	9 mo.		273.1274	14.480	0.0053
421	Male	9 mo.		207.5462	10.685	0.0051
425	Male	7 mo.		258.4294	12.547	0.0049
Average				211.8532	10.780	0.0051

TABLE IX. THE EFFECT OF REPRODUCTION ON THE IRON CONTENT OF FEMALE RATS

Rat No.	Age in days	History	Weight of body minus alimentary tract	Iron in body	
			gms.	mgs.	per cent
112	130	Not pregnant	101.51	4.524	0.0044
252	160	Not pregnant	116.95	6.136	0.0052
309	220	Not pregnant	151.10	8.225	0.0054
156	120	Not pregnant	188.05	9.902	0.0053
302	200	Not pregnant; 30 days after weaning litter	139.05	7.233	0.0052
313	220	Pregnant; 4 embryos	173.42	9.578	0.0055
103	130	Killed at birth of 7 young	114.61	3.561	0.0031
267	180	Killed at birth of 6 young	138.64	6.378	0.0046
157	120	Killed at birth of 8 young	164.82	7.203	0.0044
135	120	Killed at birth of 6 young	146.89	6.519	0.0044
78	130	Killed after eating new-born litter	143.00	5.617	0.0039
244	160	Killed after weaning litter	138.98	8.797	0.0063

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A STUDY OF THE UTILIZATION OF THE IRON OF MEATS AS COMPARED WITH OTHER PROTEIN FOODS*

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INTRODUCTION

THIS paper is a progress report of an investigation of the utilization of the iron of protein foods for growth and other normal functions of albino rats, the especial purpose of which was the development of evidence as to the specific values of meats, in comparison with other protein foods, as source of iron in human nutrition.

Throughout this study the basis of judgment as to the utilization of iron was the iron content of the entire body of the experimental subjects, except that the alimentary tract was removed and discarded, as the only practicable means of obviating the danger of contamination of the sample by food residues.

In a sense such a total iron determination may be considered a gross criterion—in comparison, for instance, with determinations of blood constants—but in spite of its appearance of crudity it was adopted as the basis of comparison in this study with the feeling, on the part of the writers, that it is in reality a critically significant datum, for the intended purpose, in that it precludes all possibility of evasion of a straightforward answer, on the part of the experimental subject—through redistribution of the iron of its body—to the question implied by the experimental procedure.

It is true that there are difficulties in the quantitative estimation of very small amounts of iron in the presence of relatively large amounts of calcium phosphate and other constituents of the ash of the animal body, but these difficulties were satisfactorily dealt with, as is shown in Table I.

All iron estimations were made, in a solution of the ash of the sample, by an adaptation of the colorimetric thiocyanate method as improved by Marriott and Wolf (1).

The analytical procedure, in brief outline, is as follows:

Dissolve the ash in 4 N HCl. To make a satisfactory solution of the

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ash of a large rat requires 200 c.c. of the acid; while 25 c.c. is sufficient for the ash of such a sample as 2 to 3 grams of muscle. A clear solution which is not too concentrated, is required.

For the iron estimation take a 1 to 5 c.c. portion of the ash solution,—the volume depending on the quantity of iron present—the smaller quantity of the more concentrated solutions being employed.

Place the aliquot of the ash solution in a 25 c.c. volumetric flask; add a few drops of dilute $KMnO_4$, to insure complete oxidation of the iron; and make up to volume with a solution prepared by dissolving 10 grams of NH_4CNS in 45 c.c. of water and 200 c.c. of acetone.

Compare the resulting red solution, in the colorimeter, with known standard iron solutions, made with 4 N HCl, and treated in all respects in the same manner as was the unknown solution.

TABLE I. SHOWING THE COMPLETENESS OF RECOVERY OF KNOWN QUANTITIES OF IRON ADDED TO SOLUTIONS OF THE ASH OF LIVER AND OF THE ENTIRE BODY OF A RAT

Solution	Total iron present	Total iron found	Iron added	Iron recovered
	mg.	mg.	mg.	mg.
Liver ash	0.095	0.095	0.050	0.050
" "	0.095	0.094	0.050	0.049
" "	0.145	0.146	0.100	0.101
" "	0.145	0.142	0.100	0.097
" "	0.095	0.096	0.050	0.051
Rat ash	0.179	0.179	0.050	0.050
" "	0.179	0.179	0.050	0.050
" "	0.224	0.221	0.100	0.097
" "	0.224	0.226	0.100	0.102
" "	0.177	0.179	0.050	0.052
" "	0.177	0.179	0.050	0.052
" "	0.176	0.178	0.050	0.052
" "	0.176	0.178	0.050	0.052

SCHEDULE OF EXPERIMENTATION

The scope and general outline of this study are set forth in Table II, there being three series of experiments, involving a total of 74 rats, fed in 15 lots, usually of five rats each.

In the greater part of the work the rats were fed individually, with control of both quantity and composition of the rations, for the maintenance of valid conditions for the comparison of results.

Series 1 was designed to reveal the specific effects of skim milk and of whole milk on the iron content of the body; the purpose of Series 2 was

TABLE II. SCHEDULE OF EXPERIMENTATION

	Diet No.	Number of rats	Duration of experimental feeding	Protein Foods	Dietary treatments General character of diets
Series 1	Diet No. 2	5	6 weeks	Skim milk	Diets designed to reveal the specific effect of skim milk and of whole milk on the iron content of the body.
	" " 2	7	9 weeks	Skim milk	
	" " 16	5	5 weeks	Whole milk	
Series 2	Diet No. 8	5	5 weeks	Beef muscle	Diets designed to reveal the specific effects of protein foods on the iron content of the body; each ration containing 16 per cent of protein, and the naturally accompanying iron, from the protein foods indicated, there being no other source of either protein or iron.
	" " 10	5	5 "	" kidney	
	" " 11	5	5 "	" brain	
	" " 12	5	5 "	" liver	
	" " 17	5	5 "	Navy beans	
	" " 20	4	5 "	Hen's egg	
	" " 22	5	5 "	Peanuts	
Series 3	Diet No. 13	4	5 weeks	Beef muscle	Diets as in Series 2, except that their iron contents were equalized by the addition of ash of the protein foods.
	" " 14	5	5 "	" brain	
	" " 15	5	5 "	" liver	
	" " 19	5	5 "	Navy beans	
	" " 21	4	5 "	Hen's egg	

to determine the specific effects of muscle, kidney, brain, liver, kidney beans, hen's egg, and peanuts, likewise on the iron content of the body, each of these foods being used to supply 16 per cent of crude protein, and the naturally accompanying iron, in a diet containing no other protein or iron, while Series 3 was designed in the same way as was Series 2, except that the iron contents of the diets were equalized by the incorporation in each of a sufficient quantity of the ash of its protein food to bring the iron content of the diet as a whole to 0.0074 per cent.

Details of Experimentation

The rats used as subjects were 24 to 28 days of age, and weighed from 40 to 50 grams each at the beginning of the experimental feeding, these animals having been reared on the stock diet described in the preceding paper.

During the experimental feeding the rats were confined in cages with false bottoms of galvanized woven wire, and, except for the rats which received diet No. 2 in Series 1, were fed individually.

The quantities of food consumed were recorded in all cases; and in Series 2 and 3 each animal received approximately the same quantity of food in relation to its live weight, the rate of feeding being determined by the animals which received the least palatable ration,—this rate being one which supplied as much food as these animals which received the least palatable ration could be depended upon to consume.

Table III. Numerical Basis of Control of Food Intake

Diet No.	Protein food	Grams of food eaten per week, per gram of body weight				
		1st week	2nd week	3rd week	4th week	5th week
16	Whole milk powder	.68	.55	.38	.47	.38
8	Beef muscle	.80	.58	.37	.48	.36
10	Beef kidney	.86	.56	.37	.45	.36
11	Beef brain	.69	.61	.41	.49	.37
12	Beef liver	.60	.55	.37	.47	36
17	Beans	.77	.55	.47	.41	.37
22	Peanuts	.54	.46	.42	.47	.40
20	Eggs	.66	.52	.38	.46	.37
13	Beef muscle + ash	.66	.49	.38	.35	.30
14	Beef brain + ash	.55	.55	.38	.46	.34
15	Beef liver + ash	.63	.57	.37	.46	.38
19	Beans + ash	.60	.62	.43	.49	.36
21	Eggs + ash	.57	.52	.38	.43	.31

In Table III is given the numerical basis of the control of food intake. At the beginning of the experiments the rats had just been weaned, and the first week of experimental feeding was the first full week during which the rats had lived on solid food alone. During this week, therefore, the food consumption was necessarily *ad libitum*. Beginning with the second week, and continued during the remainder of the experiment, the food was weighed out to each rat individually each day, at the rate in relation to live weight at which it was consumed by the rats which received the least palatable diet—except as compromises in this order were imposed by irregularities of behavior of the animals.

At the termination of the five weeks' experimental feeding each rat was killed by the use of chloroform; the alimentary tract was removed and discarded; the body was put into a porcelain dish and dried in a Freas oven; and was slowly ashed in an electric muffle furnace, preparatory to the determination of iron by the method previously outlined.

Experimental Series 1

The diets fed in this series were as indicated in Table IV, the iron and the protein being supplied by skim milk powder in diet No. 2 and by whole milk powder in diet No. 16.

Table IV. Composition of Diets. (Parts per 100)*

Diet No.		2	16
Skim milk Powder		50.00	—
Whole milk Powder		—	64.84
Starch		34.95	—
Sucrose		—	25.92
Crisco		9.00	—
Agar agar		2.00	—
Dried yeast		1.00	* —
Cod liver oil		2.00	—
NaCl		1.00	1.00
$CaCO_3$		—	1.00
$MNSO_4-4H_2O$, NaF, $AlK(SO_4)_2-12H_2O$, Na_2SiO_3	each .0125 per cent	.05	—
Iron content (per cent)		0.0015	0.0020

* Cod liver oil and yeast were fed three times per week with diet No. 16.

The inclusion of the half per cent of inorganic salts, other than sodium chloride, indiet No. 2, was suggested by the work of Daniels and Hutton (2), of Sure (3), and of Mitchell and Schmidt (4), the last mentioned workers having produced a nutritional anemia by the feeding of a diet containing these inorganic salts, without added iron.

TABLE V. THE IRON CONTENT OF THE BODIES OF ALBINO RATS AS AFFECTED BY DIETS IN WHICH PROTEIN AND IRON WERE SUPPLIED BY SKIM MILK OR BY WHOLE MILK ALONE

Rat No.	Diet No.	Number of weeks of feeding	Source of protein and iron	Av. daily gain in body weight	Av. daily food intake	Av. daily iron	Iron content of body minus alimentary tract	
				grams	grams	mgs.	mgs.	per cent
160	16	5	Whole milk powder	1.71	4.94	0.099	2.010	.0021
161	16	5		1.66	5.03	0.101	2.447	.0026
162	16	5		1.43	4.37	0.087	1.990	.0023
163	16	5		1.71	4.80	0.096	1.810	.0019
164	16	5		1.63	4.89	0.098	2.000	.0022
Average				1.63	4.81	0.096	2.051	.0022
57	2	6	Skim milk powder				2.444	.0026
59	2	6					2.084	.0025
61	2	6					1.700	.0024
63	2	6					1.902	.0023
65	2	6					2.150	.0025
Average							2.056	.0025
15	2	9	Skim milk powder				2.150	.0019
16	2	9					3.093	.0024
25	2	9					2.471	.0020
26	2	9					2.823	.0022
31	2	9					2.354	.0021
37	2	9					2.544	.0023
32	2	9					2.977	.0024
Average							2.630	.0022

Diet No. 2 was fed *ad libitum* to groups of 5 and 7 rats for 6 and 9 weeks, respectively; and diet No. 16 was fed for 5 weeks to 5 rats, caged individually, the food intake being controlled to be the same as in Series 2 and 3.

The growth curves of the rats in the experiments of Series 1 are given in Figure 1, and the iron content of their bodies in Table V.

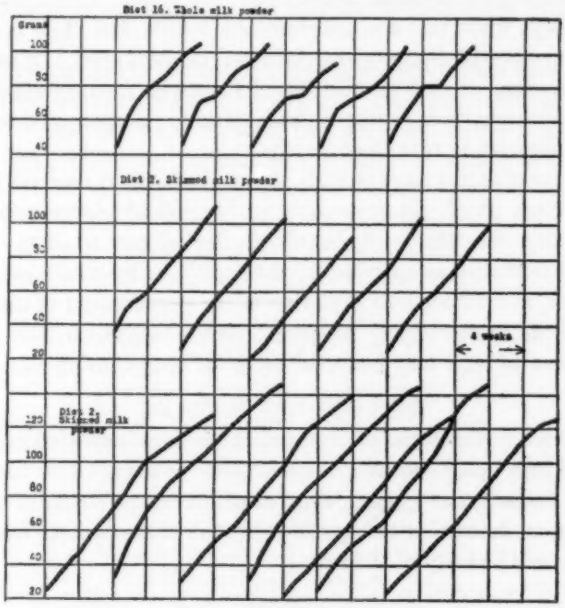

FIGURE 1

There was a striking difference between the percentage iron contents of the bodies of the rats of this series of experiments, and of normal rats, as observed by Smythe and Miller (5),—the bodies of these rats which received protein and iron only in the form of skim milk powder, or whole milk powder, containing about one-half as much iron as normally do rats of the same age and weight.

There were slight differences between the iron contents of the bodies of the rats which received diet No. 2 (skim milk) for six weeks as compared with those which received the same diet for nine weeks,—indicating a slight increase in the iron impoverishment of the group fed for nine weeks, during their last three weeks on this diet; and with diet No. 16 (whole milk) the iron depletion was a little more rapid than with diet No. 2, since the iron content of the rats which received diet No. 16 for five weeks was

slightly lower than that of the rats which received diet No. 2 for six weeks, and was the same as that of rats which received diet No. 2 for nine weeks.

It is noteworthy that the iron content of the bodies of the rats of this series of experiments is about the same as that of normal rats about 20 days of age, which have been considered by Smythe and Miller (5) as in a state of normal physiological anemia.

Presumably, when rats are continued on a milk diet after the suckling period, this state of iron impoverishment is continued. However, the continued feeding of rats on diet No. 2 for seven months resulted in no noticeable abnormalities except a characteristic paleness of the skin, and a failure of females to rear their young.

Several investigators have already shown that milk is markedly deficient in those factors which are necessary for hemoglobin formation; and the present finding that the confinement of growing rats to diets containing protein and iron only as supplied by skim milk, or by whole milk, results, in five weeks, in a 50 per cent depletion of the iron content of the body (on the basis of the fresh weight), suggests that the same nutritive deficiency, or deficiencies, of milk may be responsible for both conditions.

Experimental Series 2.

The diets fed in this series of experiments were as indicated in Table VI, protein and iron being contained therein only as supplied by beef kidney, liver, brain, muscle, navy beans, eggs, or peanuts; the object of the experiments being to determine the specific effect of these protein foods on the iron content of the body of the rat.

Table VI. Composition of Diets. (Parts per 100)*

Diet No.	8	10	11	12	17	20	22
Dried beef muscle	24.53	—	—	—	—	—	—
" " kidney	—	24.92	—	—	—	—	—
" " brain	—	—	34.71	—	—	—	—
" " liver	—	—	—	27.04	—	—	—
" beans	—	—	—	—	65.07	—	—
" eggs	—	—	—	—	—	36.05	—
Peanuts	—	—	—	—	—	—	60.67
Sucrose	64.32	62.74	63.29	57.68	17.38	60.52	27.33
Crisco	9.15	10.34	—	13.28	15.55	1.43	—
NaCl	1.00	1.00	1.00	1.00	1.00	1.00	1.00
$CaCO_3$	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Iron content (per cent)	.0028	.0074	.0043	.0052	.0063	.0033	.0011

* Cod liver oil and yeast were fed three times per week with the above diets.

The several meats used were purchased locally, and were prepared without contact with iron. As much fat was removed from the lean tissue, as was practicable, by the use of a knife of nearly iron-free phosphor-bronze, and the raw lean material was ground in a food chopper, also constructed entirely of nearly iron-free phosphor-bronze. The ground meat was then placed in a large porcelain dish, and was slowly dried in a Freas oven at 85° C.

The beans were slowly cooked in water until tender; the water was then evaporated off, and the beans slowly dried. The eggs were beaten before drying. The peanuts were roasted, but were not salted. All of these materials, when dried, were ground in the phosphor-bronze food chopper.

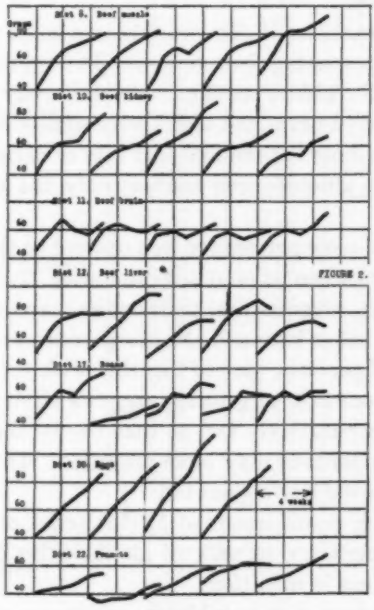

FIGURE 2

The rats were caged and fed individually during five weeks.

The food intake of the various groups was approximately the same in relation to live weight, the allowance being equal to the consumption of diet No. 8, which was fed *ad libitum*. Each diet contained 16 per cent of crude protein.

The growth curves in Figure 2, show that except for those rats which received eggs, which made excellent growth, the rats of this series did not grow as well as did those of Series 1. The growth of the rats which received

muscle, liver, or kidney was only fairly rapid; while the growth of the rats which received brain, peanuts, and beans was poor.

However, as is seen in Table VII, except in the case of the lot which received peanuts, the percentage iron content and the absolute quantity of iron, in all of the groups of this series, was much higher than in Series 1; and in most cases were of the general order of magnitude of these values for normal rats. In spite of considerable differences in the iron content of the diets, the percentage iron content of the bodies of the rats was fairly uniform for all of the groups of the series, except for those which received eggs or peanuts. In the case of the beef muscle lot the percentage iron content of the bodies was slightly but uniformly lower than with the rats of the other meat groups.

There was more variation in the absolute iron content of the rats of this series than in their percentage iron content. The figures for the rats which received liver, muscle, kidney, or eggs, were uniformly high; and those for the rats which received brain and beans were somewhat lower, but nevertheless higher than those for the milk-fed rats of Series 1,—the last mentioned exceeding in absolute iron content only the rats which received peanuts.

The comparatively low percentage iron content of the bodies of the rats which received egg, when viewed together with the high absolute iron content of these rats, suggests that their bodies may have been only fatter than were those in other lots; and the low percentage iron content of the rats which received peanuts, viewed together with the low iron content of the diet and the poor rate of growth, suggests either that the bodies of these rats were comparatively fat, or that the intake of iron was insufficient,—or both conditions may have prevailed. To casual observation, however, these rats did not appear fat.

The most striking result obtained in this series of experiments is the great apparent superiority to milk of all the other seven protein foods used as sources of iron in nutrition.

The conditions were unfavorable for close comparisons among the several groups comprising Series 2, because of the differences in the growth of the animals—especially because of the poor growth of the lots which received brain, beans, or peanuts—and because the investigation did not cover determinations of the fat and moisture contents of the bodies of the rats.

Experimental Series 3.

The diets fed in this series of experiments were as indicated in Table VIII, protein and iron being contained therein only as supplied by muscle, brain, liver, navy beans, or hen's eggs,—the object being to de-

TABLE VII. THE IRON CONTENT OF THE BODIES OF ALBINO RATS AS AFFECTED BY DIETS IN WHICH PROTEIN AND IRON WERE SUPPLIED BY SINGLE PROTEIN FOODS

Rat No.	Diet No.	Source of protein and iron	Av. daily gain in body weight	Av. daily food intake	Av. daily iron intake	Iron content of body minus alimentary tract	
			grams	grams	mgs.	mgs.	per cent
123	8	Beef muscle	1.06	4.37	0.122	3.333	.0042
124	8		1.00	4.71	0.132	3.468	.0045
125	8		1.20	3.89	0.109	3.192	.0041
126	8		1.23	4.34	0.122	3.468	.0045
127	8		1.20	4.74	0.133	3.974	.0044
Average			1.14	4.41	0.124	3.487	.0043
139	10	Beef kidney	1.20	4.26	0.315	3.593	.0047
140	10		0.80	3.63	0.269	3.209	.0049
141	10		1.43	4.43	0.328	3.750	.0049
142	10		0.94	3.83	0.283	3.297	.0052
143	10		0.83	3.57	0.264	3.061	.0051
Average			1.04	3.94	0.292	3.382	.0050
128	11	Beef brain	0.60	4.09	0.176	3.277	.0055
129	11		0.49	4.06	0.175	2.705	.0048
130	11		0.57	3.86	0.166	2.817	.0051
131	11		0.49	3.86	0.166	2.784	.0054
132	11		0.80	3.86	0.166	2.733	.0043
Average			0.59	3.95	0.170	2.863	.0051
134	12	Beef liver	0.80	4.54	0.236	3.743	.0050
135	12		1.09	4.49	0.233	4.000	.0046
136	12		0.74	3.83	0.199	3.637	.0044
137	12		0.80	4.71	0.245	3.297	.0049
138	12		0.60	4.09	0.213	3.704	.0048
Average			0.81	4.33	0.225	3.682	.0047
165	17	Beans	0.89	4.29	0.270	3.243	.0049
166	17		0.49	3.37	0.212	2.609	.0052
167	17		0.63	4.14	0.261	2.927	.0047
168	17		0.37	3.83	0.241	2.765	.0050
169	17		0.60	4.20	0.265	2.703	.0047
Average			0.60	3.97	0.250	2.849	.0049
185	20	Eggs	1.23	3.26	0.108	2.741	.0035
186	20		1.49	3.77	0.124	3.166	.0037
187	20		1.97	4.66	0.154	4.125	.0040
188	20		1.49	4.40	0.145	3.141	.0037
Average			1.55	4.02	0.133	3.293	.0037
193	22	Peanuts	0.43	3.00	0.033	1.506	.0030
194	22		0.29	2.37	0.026	1.182	.0028
195	22		0.60	3.29	0.036	1.471	.0028
196	22		0.34	3.49	0.038	1.829	.0034
197	22		0.63	3.43	0.038	1.847	.0031
Average			0.46	3.12	0.034	1.567	.0030

termine the specific effect of these protein foods on the iron content of the body of the rat; each diet containing 16 per cent of protein, supplied by one of the protein foods mentioned; and the iron content of these diets being equalized by the addition in each case of such quantity of the ash of the protein food used as required to make the iron content of the diet 0.0074 per cent,—the quantity present in the kidney diet (Series 2, No. 10) without any such ash addendum.

TABLE VIII. COMPOSITION OF DIETS. (PARTS PER 100)*

Diet No.	13	14	15	19	21
Dried beef muscle	24.53	—	—	—	—
" " brain	—	34.71	—	—	—
" " liver	—	—	27.04	—	—
" beans	—	—	—	65.07	—
" eggs	—	—	—	—	36.05
" beef muscle ash	1.69	—	—	—	—
" " brain ash	—	1.70	—	—	—
" " liver ash	—	—	0.51	—	—
" bean ash	—	—	—	0.50	—
" egg ash	—	—	—	—	1.39
Sucrose	64.32	63.29	57.68	17.38	60.52
Crisco	9.15	—	13.28	15.55	1.43
NaCl	1.00	1.00	1.00	1.00	1.00
$CaCO_3$	1.00	1.00	1.00	1.00	1.00
Iron content (per cent)	.0074	.0074	.0074	.0074	.0074

* Cod liver oil and yeast were fed three times per week with the above diets.

In most respects the results obtained in Series 2 and 3 were closely similar; and in the latter there was no indication of the utilization of the iron added to the diets in the form of ash of the protein foods. The growth of these rats, as is shown in Figure 3, was about the same as in Series 2.

The iron content of the bodies of the rats in both series is much the same, except that in Series 3 the percentage iron content of the rats which received brain and brain ash was somewhat lower than in the case of those which received brain in Series 2; and the absolute amount of iron in the rats which received liver and liver ash was considerably lower than that in the rats which received liver in Series 2.

The outstanding result in this series, which confirms a similar result obtained in Series 2, is the low percentage iron content of the bodies of the rats which received egg, which, as previously observed, may signify only that these rats, which grew very well, were fatter than others.

In the case of the rats of Series 1, which received protein and iron only as skim milk or as whole milk, the very low iron content of the bodies was

in harmony with the extensive evidence in the literature signifying that milk is characteristically poor in the nutritive requirements of the blood; and the extremely low iron content of the diet which contained peanuts, considered together with the low body content of iron, suggests that with

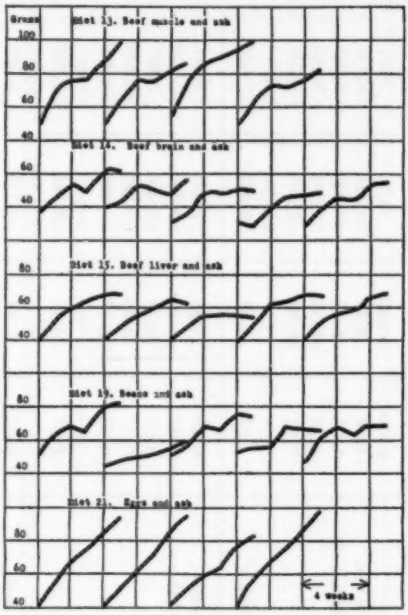

FIGURE 3

the rats which received this diet there was also nutritional anemia; but the observations made were insufficient to show the extent to which the apparent specific effects of the protein foods on the iron contents of the bodies of the rats were determined by differences in growth and in the fatness of the bodies of the rats.

These studies are being continued in experiments in which the iron contents of the bodies of the rats will be reckoned to the fat-free and moisture-free basis.

SUMMARY

In a study of the specific effects of meats and other protein foods on the iron content of the growing albino rat 74 individuals were fed, in most cases individually, from the time of weaning for periods usually of 5 weeks, after which iron was quantitatively determined in the entire bodies minus the alimentary tract.

The following protein foods were compared: Skim milk powder, whole

TABLE IX. THE IRON CONTENT OF THE BODIES OF ALBINO RATS AS AFFECTED BY DIETS IN WHICH PROTEIN AND IRON WERE SUPPLIED IN CONTROLLED AMOUNTS BY SINGLE PROTEIN FOODS

Rat No.	Diet No.	Source of protein and iron	Av. daily gain in body weight	Av. daily food intake	Av. daily iron intake	Iron content of body minus alimentary tract	
			grams	grams	mgs.	mgs.	per cent
170	13	Beef muscle and	1.34	4.29	0.317	3.792	.0044
171	13	beef muscle ash	1.03	4.49	0.332	3.828	.0043
173	13		1.23	4.34	0.321	3.449	.0043
174	13		0.91	4.03	0.298	3.556	.0048
Average			1.13	4.29	0.317	3.656	.0045
144	14	Beef brain and	0.69	4.34	0.321	3.371	.0048
145	14	beef brain ash	0.46	4.06	0.300	2.830	.0044
146	14		0.51	4.14	0.306	2.857	.0047
147	14		0.51	3.86	0.286	2.728	.0044
148	14		0.69	3.97	0.294	3.077	.0044
Average			0.57	4.07	0.301	2.953	.0045
155	15	Beef liver and	0.80	3.74	0.277	3.023	.0050
156	15	beef liver ash	0.66	3.49	0.258	2.856	.0046
157	15		0.34	3.37	0.249	2.538	.0055
158	15		0.80	3.69	0.273	2.686	.0045
159	15		0.77	3.66	0.271	2.956	.0049
Average			0.67	3.59	0.266	2.812	.0049

TABLE IX. THE IRON CONTENT OF THE BODIES OF ALBINO RATS AS AFFECTED BY DIETS IN WHICH PROTEIN AND IRON WERE SUPPLIED IN CONTROLLED AMOUNTS BY SINGLE PROTEIN FOODS—Cont.

180	19	Beans and bean ash	0.69	3.11	0.230	2.753	.0049
181	19		0.37	2.69	0.199	2.691	.0059
182	19		0.71	3.43	0.254	2.857	.0050
183	19		0.57	3.46	0.256	2.720	.0050
184	19		0.69	3.63	0.269	2.410	.0045
—			—	—	—	—	—
Average			0.61	3.26	0.242	2.686	.0051
189	21	Eggs and egg ash	1.46	3.71	0.275	3.373	.0039
190	21		1.54	3.63	0.269	3.201	.0037
191	21		1.17	3.31	0.245	2.674	.0036
192	21		1.66	4.03	0.298	3.219	.0037
—			—	—	—	—	—
Average			1.46	3.67	0.272	3.117	.0037

milk powder, beef muscle, beef kidney, beef brain, beef liver, navy beans, hen's egg, and peanuts.

The diets were so compounded that in each case a single protein food constituted the sole source of protein and of iron, these foods being used in quantities supplying the same amounts of protein; the iron content of each diet being that which naturally accompanied the protein food used or, in other cases, an amount controlled by supplementing the quantity of iron naturally present in a protein food by adding ash of the same product.

The meats were fed ground and dried, after preparation with the aid of a phosphor-bronze knife and meat chopper. Iron was determined by an adaptation of the colorimetric thiocyanate method of Marriott and Wolf.

The growth from diets in which the sole source of protein and iron was skim milk powder or whole milk powder was excellent, but in the course of 5 weeks' feeding the iron content of the bodies of the rats was reduced to approximately 50 per cent of the normal.

After feeding for seven months on the diet in which skim milk constituted the sole source of protein and iron, female rats were unable to rear their young.

The growth from the egg diets was excellent; from the muscle, liver, and kidney diets was fair; and from the brain, bean, and peanut diets was poor.

The iron content of the rats which recieved the meat foods was apparently normal in each case; the iron content of the rats which received egg was much lower, the iron content of egg apparently being less efficiently utilized than that of the meats; and the iron content of the rats which received peanuts being the lowest of all, the iron content of peanuts being especially low.

Each of the seven protein foods studied, other than milk, was much superior to milk as a source of dietary iron for growing rats.

There is ground for question as to the validity of the apparent specific effects of the protein foods studied, especially of egg and peanuts, by reason of the facts that the gains in body weight in the several experimental lots were very different, and that determinations were not made of the moisture and fat contents of the bodies of the rats; the existence of milk anemia, however, is certainly well established.

There was no evidence of the utilization of supplementary iron, in the form of ash of muscle, brain, liver, navy bean, and egg.

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GROWTH RATES OF BROOK TROUT REARED UPON PURIFIED RATIONS, UPON DRY SKIM MILK DIETS, AND UPON FEED COMBINATIONS OF CEREAL GRAINS*

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Introduction

In an earlier series of experiments we have shown that brook trout will grow for a period of about twelve weeks upon a purified ration if the protein which is furnished by casein is fed at a level higher than ten per cent (1). Those trout fed at a ten per cent casein level fail to grow but continue to live for nearly six months while those that grow upon the higher levels die in about half this time. Trout stunted upon low protein diets do not lose their power of growth but can resume a normal rate after twenty weeks of stunting if allowed an adequate diet. We have been unable to show that our failures in rearing trout upon purified rations are due to any known mineral or vitamin factors.

Our previous experiments yielded failures when dried liver and dried cracklings were the sole sources of protein in the rations. These failures were not due to a roughage deficiency since an addition of agar-agar yielded the same failures. Of the dry feeds included in our former studies, skim milk was very promising.

In the experiments recorded in this report we have employed the same technique as described previously (2). The relative value of a given ration is gauged by its ability to promote growth and prevent death in groups of brook trout confined in separate troughs with independent intakes and outlets for water flow. The growth curves of our charts have been constructed from weights taken at periods of two weeks. Our procedure consists of weighing whole groups, determining the average weights, and then plotting the growth curve of the average individual. All weights are expressed and plotted in grams and time in weeks. The water furnished the troughs for these experiments was piped directly from a spring. The temperature of this water is very uniform throughout

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the year. Daily determinations show it to remain quite constant at 50°F ± 2.5.

The composition of the diets that are involved in these experiments are given in Table I. Basal ration "B" was made up of casein 35%, starch 62, and salt mixture 3. This ration as well as numbers 1 and 18 was prepared by mixing the salt mixture, starch, and casein in the given pro-

TABLE I

COMPOSITION OF RATIONS EMPLOYED

No. 1	No. 1–b	No. 5
Casein....10	Raw liver....20	Dry skim milk....98
Starch....82	Diet No. 1....80	Cod liver oil.... 2
Salt mixt.... 6		Yeast
Cod liver oil.... 2		
Yeast		
No. 11	**No. 12**	**No. 17**
Raw liver....61	Raw liver....90	Lactose....50
Dry skim milk....37	Dry skim milk.... 7	Casein....39
Cod liver oil.... 2	Cod liver oil.... 2	Cod liver oil.... 2
Yeast	Starch.... 1	Salt mixt.... 9
	Yeast	Yeast
No. 18	**No. 18a**	
Casein....10	Raw liver....20	
Starch....85	Basal ration "B"....80	
Salt mixt.... 3		
Cod liver oil.... 2		
Yeast		
No. 23	**No. 24**	**No. 25**
Dry skim milk....93	Basal ration B....78	Basal ration B....83
Raw liver.... 5	Raw liver....20	Vitavos....15
Cod liver oil.... 2	Cod liver oil.... 2	Cod liver oil.... 2
Yeast	Yeast	Orange Juice
No. 26		**No. 28**
Red dog flour....65		Raw liver....100
Salt mixt. A.... 3		
Raw liver....30		
Cod liver oil.... 2		
No. 29	**No. 30**	**No. 31**
Corn gluten....35	Restricted intake of raw liver	Fish meal....24
Starch....30		Starch....41
Salt mixt. A.... 3		Salt mixt. A.... 3
Cod liver oil.... 2		Cod liver oil.... 2
Raw liver....30		Raw liver....30
No. 32	**No. 37**	**No. 38**
Peanut meal....24	Ether extract liver	Alcohol extract liver
Starch....41	Basal ration B....98	Basal ration B....98
Salt mixt. A.... 3	Cod liver oil.... 2	Cod liver oil.... 2
Cod liver oil.... 2	Yeast	Yeast
Raw liver....30		

portions while the constituents were in the form of dry powders. These were converted to a thick paste with cold water and poured with stirring into a considerable volume of boiling water. The mixture was then boiled until it was too thick to stir. It was dried in a gas oven and ground to a powder. In all other cases in which starch is included in a ration, it is employed in the uncooked state.

Two salt mixtures have been used in these experiments. The term "salt mixture" means that of Osborne and Mendel, while "salt mixture A" is a simple mineral mixture consisting of one part calcium carbonate, one part bone meal, one part sodium chloride, and a trace of potassium iodide.

Yeast was fed in an amount estimated at one half gram of yeast per day per two hundred grams of fish. The yeast employed was the dried product of the Northwestern Yeast Co.

The red dog flour, corn germ meal, corn gluten, fish meal, and peanut meal were ordinary commercial products.

All trout were reared upon the usual mixed hatchery feeds until placed upon an experiment.

Rearing Brook Trout Upon Purified Rations

In our previous reports (3) we have emphasized the importance of growth studies upon trout when rations are used whose basic composition is made up of relatively simple units such as casein, starch, mineral mixture, and vitamin concentrates. We have shown that trout display only a meagre growth upon such rations even when they are fed in proportions that promote normal growth and reproduction in the rat. This growth is followed by death after a period of about three months.

In the previous report we have also shown that trout are stunted upon diets containing ten per cent casein and either three or six per cent salt mixture. This work has been repeated and extended over a much longer period to determine whether trout lose their power of growth after long stunting. Two groups of trout were fed for a period of twenty-eight weeks upon low protein diets, Nos. 1 and 18 respectively. The average growth curves (Chart 1) of individuals of these groups show that there was no growth throughout this entire period. Since the death rate started to increase rapidly at the end of the twenty-eight weeks' period, each group was divided. Half of group eighteen were continued upon the previous diet. As shown in Chart 1, these all died in the course of another eight weeks.

Half of group one was changed to diet 1-b which is merely the previous ration No. 1 supplemented with 20 per cent raw liver. Although the members of this group had remained at an average weight of seven grams

for twenty-eight weeks, they had not lost the power of growth since in the next fifteen weeks they resumed a normal rate and attained an average weight of twenty-nine grams.

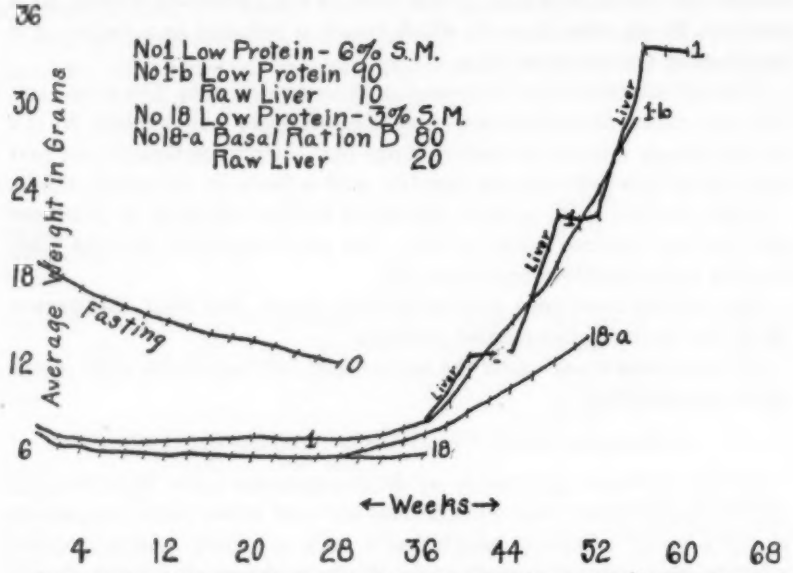

CHART 1. Growth of trout after periods of stunting and during periods of alternate growth and stunting. Weight loss during fasting.

Likewise the other half of group eighteen was transferred to diet 18-a which also permitted growth. In both cases there was some mortality after the transfer. We have constantly observed that the death rate is not immediately checked by transfer from a poor to a good ration. The growth upon rations 1-b and 18-a is shown in Chart 1. These experiments not only show that trout do not lose their power of growth after being stunted for a period of nearly seven months but also indicate that there is nothing in diets No. 1 and basal diet B which inhibits growth. Curve 24 of Chart 2 yields further evidence that basal ration B was not toxic since it permitted quite normal growth over a period of twenty weeks when it was supplemented with raw liver.

The remaining halves of groups 1 and 18 were pooled at the end of thirty weeks. In the course of the next six weeks they were gradually transferred from their previous rations to one of raw liver. For the next four weeks they were allowed to grow upon raw liver, consuming all they

wished. At the end of this time they were placed upon diet 1 for four weeks. At the end of this period they were again placed upon liver diet and allowed to grow. We have thus obtained alternate periods of growth and stunting. This is shown in the curve which is the continuation of No. 1 of Chart 1. The growing periods are marked "liver" while the stunting periods are marked "1". After the initial losses, there have been few deaths under this regime. This experiment of alternate stunting was

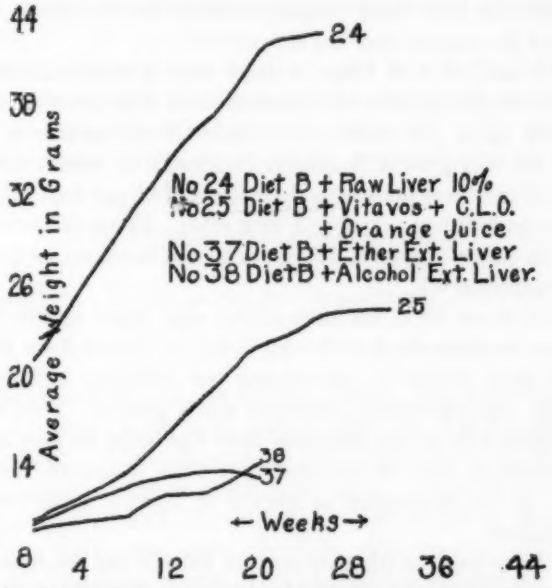

CHART 2. Growth of trout upon a basal ration supplemented with raw liver, with Vitavos and with extracts of of liver.

undertaken originally to provide an experimental proof that the rings upon the scales of fish are indices of their age. The accepted theory of the production of these rings is that they are the marks produced by alternate periods of rapid growth when food is abundant naturally, and periods of stunting when food is scarce. Since this experiment is still in progress, we must reserve the discussion of the production of rings upon the scales for a later report. These data do indicate, however, that during the periods of growth there is an actual acceleration of growth rate which practically compensates for the period of stunting.

This growth acceleration confirms the earlier observations of Osborne

and Mendel (4) in their work with rats. Trout are superior to rats for such studies, however, since they can withstand considerable stunting without any apparent injury.

In curve 0 of Chart 1 we have shown the weight losses for a group of fasting trout. This shows a gradual loss of weight in contrast to the constant weight of the group upon the low protein diets after the latter had suffered an initial loss. From this we may conclude that those trout fed the low protein rations do not fast but consume just about enough food to about maintain their body weights without any increase. It appears that trout eat for calories and not for protein.

Curves 1-b and 18-a of Chart 1 show that growth can be obtained upon purified rations if these are supplemented with about a fourth their weight of raw meat. In order to determine if the portion of raw meat responsible for this growth is soluble in alcohol or ether, we extracted five pounds of raw liver pulp with three liters of 95 per cent ethyl alcohol. Another five pounds were extracted with ether. These extracts were then evaporated in vacuo upon two 750 gram lots of basal ration B at a maximum temperature of 65° C.

In Chart 1, curve 18-a, we have shown that basal ration B produces growth when supplemented with raw liver. In Chart 2 we have shown the growth upon ration 37, containing the ether extract, and 38, the alcoholic one. Neither group displayed much growth. That shown is of the same magnitude as that obtained upon the basal rations alone. From this experiment it must be inferred that little if any of the substance responsible for the promotion of growth in trout is extracted by either ether or alcohol.

In order to determine whether normal growth can be secured by employing a very large amount of Vitavos in conjunction with a purified ration, we have fed diet No. 25. This growth curve is reproduced in Chart 2. This cannot be termed a "purified ration" since it contains 15 per cent Vitavos. We have obtained a moderate growth rate over a period of twenty-eight weeks. This indicates again that the basal ration B is not toxic and affords a starting place for improved experiments with purified rations.

Dry Skim Milk as Trout Feed

In our earlier reports the data indicate that dry skim milk is a promising feed for trout. Our preliminary experiments showed that milk alone will produce a good growth for a period of about three months. This is followed by the very rapid death of all members of the group.

Since we felt that our earlier failures with dry skim milk alone may have been due to some inorganic factors such as those which recent workers (5) have shown produce nutritional anemia in rats and rabbits, we designed rations Nos. 33, 34, 35, and 36. These were also designed to answer the following questions: (1) Is raw spleen as satisfactory as raw liver as a supplement for dry skim milk? (2) Is dry buttermilk with its high content of lactic acid superior to dry skim milk? (3) Can the growth experiments upon trout with a given ration one year, be duplicated with the same ration but with fingerlings hatched from a new stock of eggs a year later?

The composition of rations 33, 34, 35, and 36 is shown in Table II. Into each ten pounds of dry skim milk and dry buttermilk employed in these experiments were mixed 0.15 gram of zinc sulfate, 0.20 gram of copper sulfate, 0.065 gram of potassium iodide, and 0.60 gram of ferric citrate.

TABLE II

COMPOSITION OF RATIONS EMPLOYED

No. 33	No. 34	No. 35	No. 36
Dry Buttermilk..85	Dry buttermilk...85	Dry skim milk...85	Dry skim milk...85
Vitavos.....10	Raw spleen.....15	Vitavos.....10	Raw spleen.....15
Cod liver oil..... 5		Cod liver oil..... 5	

Both the growth curves and mortality rates upon these diets are shown in Chart 3. These show quite plainly that raw spleen is detrimental to growth upon dry skim milk and dry buttermilk but that the inclusion of the spleen lowers the death rate in both cases. Dry buttermilk is slightly superior to dry skim milk when fed alone. The curves for both the growth rate and the death rate upon dry skim milk are very similar to those included in a previous report (2) and show that nutrition experiments upon trout can be repeated and duplicated in succeeding seasons upon fingerlings hatched from a new supply of eggs obtained a year later. These curves also show that the failure upon dry skim milk is not due to the lack of traces of certain minerals nor to vitamins A, B, or D but to some other factor which we have previously termed H. This conclusion is confirmed further by the fact that we have supplemented these diets with Vitavos, thus providing an entirely new source of vitamin B factors. These curves furnish a comparison with the experiments of the preceding year in which we employed yeast whose potency had been determined by growth experiments upon rats. In spite of these alterations and additions to our dry skim milk ration, no improvement was effected.

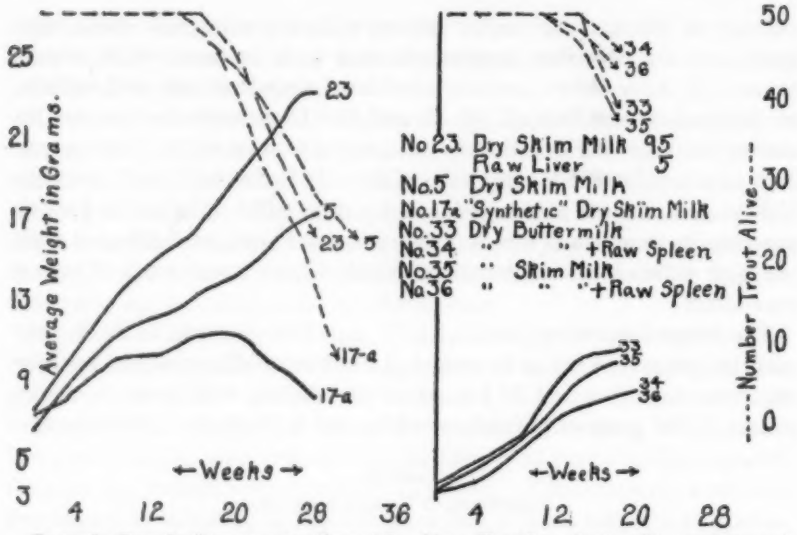

CHART 3. Growth of trout upon rations of dry skim milk and dry buttermilk, supplemented with traces of iron, zinc, copper and iodine. Broken lines show mortality rates.

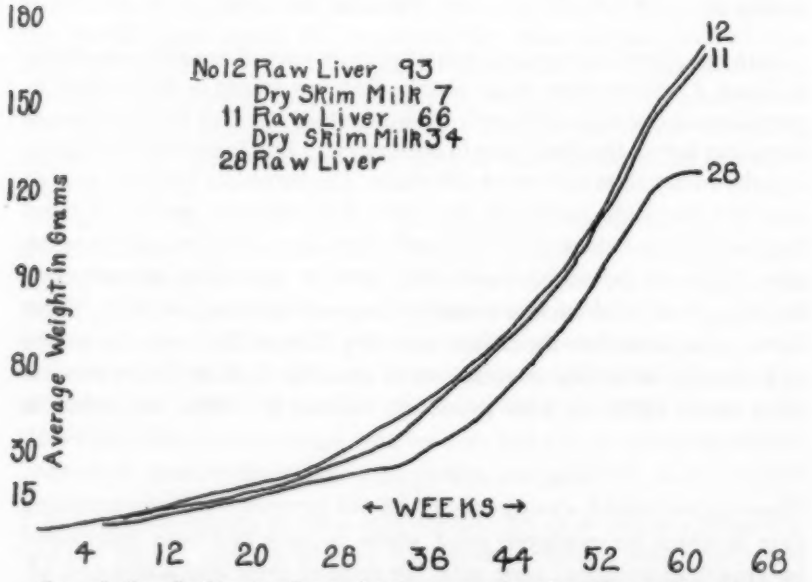

CHART 4. Growth of trout until spawning time upon rations of raw liver, and dry skim milk with raw liver.

In Chart 4 we have presented the data from a year of experiments upon dry skim milk. Group 28 which was fed raw liver served as a control for what may be considered an optimum hatchery diet. Groups 11 and 12 were fed upon a mixture of raw liver and dry skim milk in the proportions indicated in Table I of compositions of rations. After these groups, numbers 11, 12, and 28, had increased in size, their numbers were reduced from the original fifty per group to twenty-five and at the same time they were transferred to large troughs to allow adequate room to rear them to maturity. There were no death losses due to the ration. The cut in the size of the groups is not shown in the chart. No selection was exercised in dividing the groups.

In both cases the growth upon combinations of raw meat and dried milk was superior to that upon liver alone. At the end of sixty-one weeks as is shown in Chart 4, curves 11, 12, and 28, those reared upon meat-milk combinations averaged 165 grams, while those reared upon raw liver averaged about 126.

In Chart 3, curves 17-a, 5, and 23 show the relative growths and death rates obtained upon a ration of dry skim milk containing 5 per cent raw liver by weight, dry skim milk alone, and a third one composed of casein, lactose, and salt mixture in the proportions present in dry skim milk. Good growth was obtained upon the dry skim milk supplemented with 5 per cent raw liver. The first death occurred upon this diet during the sixteenth week after the experiment was started. During this period the average member of the group had increased from seven to sixteen grams. This indicates that under ordinary hatchery conditions trout could be reared for a period of three to four months upon dry skim milk with only a very small addition of raw meat. Such a procedure would be of questionable value, however, since additional safety may be procured by increasing this amount of raw meat slightly. The growth of group 5 is good but shows that dry skim milk alone is inferior to that obtained with a supplement of a small amount of raw liver. Group 17-a is markedly poor, confirming our previous conclusion that the synthetic milk made up in the laboratory is decidedly inferior to natural dry skim milk although the chemical composition is similar.

While not strictly comparable, due to the differences in the initial weights of the trout, curves 23 and 36 afford striking contrasts in the relative value of raw spleen and raw liver when employed as supplements to rations of dry skim milk for trout feeding. Raw liver in an amount as small as five per cent produces a marked acceleration in growth while raw spleen at a level of fifteen per cent has a slightly detrimental influence.

That spleen is digested in quite a different manner from liver is confirmed by the large amount of white fibrous tissue which is seen excreted from trout fed exclusively upon a diet of the former. A common hatchery trick is to feed a long strip of spleen which is later seen protruding simultaneously from the mouth and anus.

The Use of Corn Germ Meal, Red Dog Flour, Corn Gluten, Fish Meal, and Peanut Meal as Trout Feeds

In these initial studies of the protein concentrates that are cheap and commercially available, we have planned our rations so that all include the same per cent of protein. Each ration also contains mineral and vitamin supplements. A simple mineral mixture was employed. This renders it relatively cheap.

Since it has been questioned whether such concentrates as corn gluten meal can have any nutritive value for trout, we were obliged to adopt a special method of testing. If we feed one group of trout, as number 29, with a ration of corn gluten, mineral mixture, vitamin supplement, and raw liver and obtain a moderate growth, we may attribute this entirely to the allowance of raw meat and assume the other materials are inert. To check this possible error we can weigh the food consumed by No. 29 of Table I as carefully as possible. The following week a check group can be fed the same amount of raw liver that was consumed by No. 29 during the preceding week. This allowance of raw liver must be adjusted to account for differences in the weights of the two groups. In case the growth of group 29 is to be attributed only to the liver they consume, the check group receiving that amount of liver minus the other ingredients should show the same growth rate. The check group of these experiments is number 30.

While the question of whether or not trout can employ the usual cereal grains for growth and maintenance may seem ludicrous to one whose experience has been restricted to the higher mammals, it is an important practical question to the fish culturist. And it becomes a legitimate consideration when one recalls the exceedingly short intestinal tract of the trout.

Table I contains the composition of the diets. Group 26 was fed a ration containing 57 per cent red dog flour; group 27 received one containing 65 per cent corn germ meal; number 29 was fed 30 per cent corn gluten; number 31 received 21 per cent fish meal; number 32 was fed a ration containing 21 per cent peanut meal.

The growth curves for both group 30 and those groups fed upon the various proteins, chiefly of cereal origin, are shown in Chart 5. All these experiments were started with fifty members in each experimental group but since this number became too large for the available troughs they

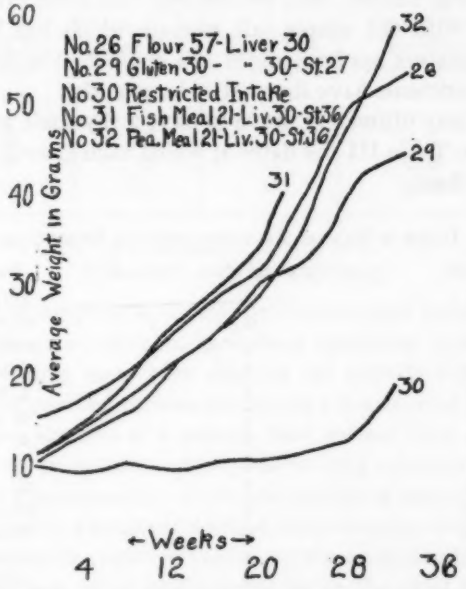

CHART 5. Growth of trout upon rations of flour, corn gluten, peanut meal and fish meal supplemented with raw liver. Stunting with a restricted intake of raw liver.

were reduced to ten in each group. This reduction was effected with a selection that produced no alteration in the average weight. In the process of reducing the numbers, group 31 was accidentally destroyed. Therefore this curve terminates while the other experiments are still in progress.

At the end of twenty-four weeks, group 30 had shown no growth. This demonstrates quite clearly that trout can use some portion of cereal grains for either growth or maintenance. Both groups 26 and 29 displayed excellent growth. At the end of twenty-four weeks group 30 was changed to ration 31 and allowed all the food it wished. Growth was quickly resumed and proceeded at an optimum rate, showing once more that trout can be stunted for many weeks but do not lose their power of growth. This group will be followed with special interest since at the

time of this report the other groups are expected to spawn. The influence of stunting upon spawning will be discussed in a later paper.

Fish meal, corn gluten, red dog flour, and peanut meal are all capable of producing good growth in trout if they are supplemented with thirty per cent of raw liver. The minimum allowance of raw liver to produce a maximum growth has not been determined. All these rations have been supplemented with the simple salt mixture which has been indicated. This mineral mixture has been added as a precaution rather than because any of our experiments have shown it to be essential.

Since trout may ultimately prove to have a rhythmic growth, we have incorporated in Table III the dates at which each growth curve starts in its respective chart.

TABLE III. DATES OF STARTING EACH EXPERIMENTAL GROUP UPON THE RATION

Diet No.	Chart No.	Date
0	6	8/31/28
1	6	10/28/27
18	6	10/28/27
24	7	5/18/28
25	7	4/20/28
37	7	4/ 6/28
38	7	4/ 6/28
11	8	9/ 3/27
12	8	9/ 3/27
28	8	10/15/27
26	9	3/16/28
29	9	3/16/28
30	9	3/16/28
31	9	3/16/28
32	9	3/16/28
23	10	10/28/27
5	10	10/28/27
17-a	10	10/28/27
33	10	6/23/28
34	10	6/23/28
35	10	6/23/28
36	10	6/23/28

THE TROUT AS A CONVERTER OF MATTER

Since higher mammals at many seasons of the year must employ some portion of their food intake for the maintenance of body temperature, it is of interest to note the efficiency of a cold blooded species like the trout in the use of feedstuffs for growth. Such data are quite difficult to obtain and at best can be considered only an estimate since in feeding fish it is necessary to have the food come into contact with the water. Hence

any figures for food consumed must reflect unfavorably upon the efficiency of the trout since losses are charged against them. We have kept many records but have included only one in Table IV.

TABLE IV. FEED CONSUMED BY SPECIAL GROUPS DURING THE MONTH OF AUGUST, 1928

Diet No.	Mean Wt. Begin. Gms.	Mean Wt. End Gms.	No. of Fish observed	Gms. of Food Protein to Produce 1 Gm. Live Fish	Wt. Feed consumed Gms.
11	89	118	21	0.9	2160
28	67	97	15	0.85	1925
12	91	120	22	0.80	2310

SUMMARY AND CONCLUSIONS

Experiments attempting to define the fundamental nutritional requirements of the lower vertebrates have been continued with brook trout. Experimental groups have been stunted for periods exceeding twenty weeks by feeding purified rations containing a low level of protein and by feeding a limited amount of a protein food, as raw liver. In both cases trout will exhibit an optimum growth after long stunting when they are placed upon an adequate diet. Alternate periods of stunting and growing indicate that there is a tendency toward compensation during the growing period which makes the rate tend to exceed the normal optimum.

The factor in raw meat which seems to be the most active agent in stimulating the growth of trout is not extracted by alcohol nor ether. It is not identical with any of the known vitamins.

Synthetic purified rations of starch, casein, and salt mixture with vitamin supplements will permit a limited growth in trout if the protein level exceeds ten per cent. Trout are equally stunted when the protein level is ten per cent whether the ration contains three or six per cent mineral matter. Trout stunted upon low protein diets live twice as long as those that are allowed to grow upon similar synthetic rations with a higher protein level. This seems to indicate that their bodies contain a store of some substance that is essential for life but is consumed in growth.

Dry skim milk has been studied as a trout feed. Experiments with various traces of mineral supplements indicate that its failure is not due to deficiencies in zinc, copper, iron, or iodine. Raw liver is a much more effective supplement for a milk diet than raw spleen. Dried buttermilk is

slightly better as a trout feed than dry skim milk when each is employed singly.

Trout are able to use corn gluten, flour, and peanut meal for growth and maintenance. As a converter of matter, the trout compares very favorably with the most efficient of the higher mammals and is possibly somewhat superior.

We wish to thank both Prof. L. A. Maynard and Chester D. Tolle for assistance and advice in conducting our experiments.

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THE ABSORPTION OF GLUCOSE FROM THE ALIMENTARY TRACT OF RATS DEPRIVED OF THE VITAMIN B COMPLEX[1] [2]

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THE functional activity of the gastro-intestinal tract is dependent to some extent upon the presence of vitamins A and B in the diet. Cramer (1923) has found that the use of diets deficient in vitamin B caused atrophy of the lymphoid tissue of the ileum. In the absence of actively functioning lymphoid tissue, the assimilation and absorption of foods from the intestine is impaired. Cowgill (1926), Gross (1924), Plummer (1927), Nelson (1925) and McCarrison (1919) (1921) (1922) have found that vitamin B deprivation caused degenerative changes in the gastro-intestinal tract which prevented the normal functioning of the component parts. Several of the latter authors have observed that the alimentary rate decreased when diets deficient in vitamin B were fed to animals. At the same time there was a decrease in the tonus of the intestinal musculature. These several observations have led to the postulation of a decreased absorption from the intestine in the absence of vitamins A and B. Bergeim (1928) using his iron oxide method (1926) fed rats and guinea pigs rations deficient in vitamins A, B, C and D. He studied the digestive efficiency of normal animals and compared it with that of animals receiving inadequate diets. Little difference was observed between the ability of the two groups to digest starch, while the protein digestion values for the two groups did show some difference, being smaller for the groups receiving vitamin deficient diets. Bergeim does not believe "that these differences are great enough to support the view of an early or specific impairment of digestive function in these avitaminoses."

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[2] The preliminary part of this investigation was carried on in the Department of Vital Economics at the University of Rochester and the latter part in the Department of Agricultural and Biological Chemistry at the Pennsylvania State College. Mr. Polansky assisted in the preliminary studies and Mr. Osgood in the later studies.

Experimental

The object of the present investigation was to determine whether or not the absence of the vitamin B complex in the diet of rats had any effect upon the absorption of glucose from the gastro-intestinal tract. Cori (1925) devised a method for studying the rate of absorption of various sugars from the intestinal tract of rats, and we have followed his technique, with some modifications. The principle of this method, as stated by Cori, is as follows: "A known amount of the substance under investigation is fed by stomach tube. After a given time the rats are killed and the amount of substance remaining in the intestines is determined quantitatively. The difference between the amount fed and the amount recovered from the whole intestinal tract is then the amount of substance absorbed."

A number of years ago Rubner (1902a) and more recently Kugler (1919) used a method for studying absorption from the alimentary tract of animals similar to that devised by Cori.

Our procedure differed from that of Cori in several ways. The rats used were older and on an average weighed more than those used by Cori. Solid food was withheld, but water was left at the disposal of the rats during the fasting periods. The normal Rochester rats were fasted for 48 hours before receiving the meal of glucose, but on account of the weakened condition of the rats on vitamin B-free diets, they were fasted for 24 hours only. Fasting periods of twenty-four hours were used with all of the other groups. With the exception of the Rochester rats, one hour absorption periods were used. Excellent checks were obtained in the determination of the amounts of glucose fed to the rats. The amount of sugar adhering to the outside of the catheter after its withdrawal was found to be negligible. The amounts of glucose remaining inside the catheter after the injection showed some variation and because of this the catheters were washed out each time after use, and the glucose in the wash water determined. The various catheters used retained different amounts of glucose. Several rats were killed with the stomach tube in place to confirm its location with the tip in the stomach. The amount of reducing substances present in the intestine of rats fasted for 24 hours was found to be small and was neglected in all calculations. Twelve animals were used in this particular phase of the invesitgation and in one case only was there more than a trace of reducing matter present. When rats had been fed sugar after a fasting period of 24 hours, no reducing substances were found in the cecum at the end of a one-hour absorption period. After having removed the alimentary tract it was cut open and

washed carefully with successive portions of warm water until approximately 500 cc. of water had been used. The intestine after washing was placed in a volumetric flask and shaken with water, this being tested for the presence of glucose. No reducing substances were found to be present. Rats which were frightened and struggled when the stomach tube was being placed in position were not used. The sugar used in all of the experiments was C.P. d-glucose obtained from the J. T. Baker Company. The strength of the glucose solutions used ranged between 30 and 50 per cent, and the solutions were prepared 24 hours before using (we, of course, having accepted Cori's statement that concentration did not affect the rate of absorption). Bertrand's method was used for the quantitative determination of glucose.

None of the rats exhibited glycosuria during the absorption periods and there was evidence of diarrhoea once or twice only.

Before studying the effect of the absence of vitamin B on the absorption of glucose, a number of determinations were made using normal animals three to five months of age which had been on the colony ration (Mattill and Clayton, 1926) at Rochester. Later several groups of rats were placed on a vitamin B-free diet of the following composition: Casein (vitamin B-free) 18%, salt mixture (McCollum 185) 4%, corn starch 56%, lard 20%, cod liver oil 2%.

A record of the food intake of the Rochester group of animals on a vitamin B-free diet was not kept. These animals were placed on the deficient diet for 41 to 46 days, and at the end of this period they were in a greatly weakened condition. At the end of the diet period the rats were fasted for 24 hours, and the glucose absorption determined as in the case of normals using Cori's procedure.

In later work at the Pennsylvania State College two groups of rats were used, one group consisting of animals from the stock colony of pied rats and known as "Penn State" rats, and the other group consisting of animals obtained from the Albino Supply Company at Philadelphia, designated by the Company as "large size, toxicity" rats. The Penn State rats have proved to be very resistant to the effects of vitamin B deprivation and it has been suggested by associates of the writer at State College that a micro-organism capable of synthesizing vitamin B was a member of the intestinal flora, or that the intestinal flora was altered in some other way, so that toxic substances ordinarily formed and absorbed were absent. Wright (1921) has expressed the opinion that the explanation for the early decline in rats deprived of vitamin B lies in the fact that toxic products are formed in the abnormal length of time that food remains

in the intestine. The toxins thus formed are absorbed and affect the general health of the animal. Fridericia and his coworkers (1927) have found that a number of their young rats, receiving vitamin B-free diets, grew, thrived and multiplied. The bacterial flora in the cecum of their rats differed from that of other rats, and the reaction of the contents of the cecum was acidic instead of neutral or alkaline.

It was owing to the fact that the Penn State rats did not show the effects of vitamin B deprivation that rats were obtained from another source. This second group of animals was kept in another building in order to avoid contact with the Penn State animals.

A record of the food intake was kept for all rats placed on vitamin B-free diets at the Pennsylvania State College. Because the original deficient ration tended to be lumpy and was easily scattered the composition was changed slightly at the latter institution. Instead of 56% corn starch, 66% was used and the percentage of lard reduced from 20 to 10. Otherwise the two rations were identical. The normal Penn State and the normal Albino Supply Company rats were kept on the diet used for the breeding stock (Dutcher, et al, 1925).

Results

The results obtained with normal animals from the Rochester colony are shown in Table I. The average amount of glucose absorbed during the one- and two-hour periods was greater than that found by Cori, while the quantity absorbed during the third hour closely approximated his first results. (See also Chart 1.) Compared with those reported by Cori in two later papers (1928a) (1928b) our values for absorption during the one- and two-hour absorption periods are but slightly higher. Variations are to be noted in our results which are much greater than those found by Cori. For the one-hour absorption period the standard deviation calculated on the basis of absorption per 100 grams of body weight was 59.3 mgms. and the coefficient of variability 25.13% while on the basis of surface area the corresponding figures were 0.289 mgms. per sq. cm. and 20.60%. The standard deviation for the two-hour period on the basis of absorption per 100 grams body weight was 26 mgms. and the coefficient of variability 11.35% and the values obtained based on surface area were 0.107 mgms. per sq. cm. and 7.82%. For the three-hour period the standard deviation based on 100 grams body weight was 14.6 mgms. and the coefficient of variability 8.69%, while on the basis of surface area the standard deviation was 0.078 mgms. per sq. cm. and the coefficient of variability 7.85%. The results are more nearly uniform when

the absorption periods are longer than one hour in length. The choice of litter mates in our work did not result in values which are any more nearly uniform.

The animals in the three groups show losses in weight during the 24 hours preceding the absorption period (Table I) which are somewhat lower than those observed by Cori.

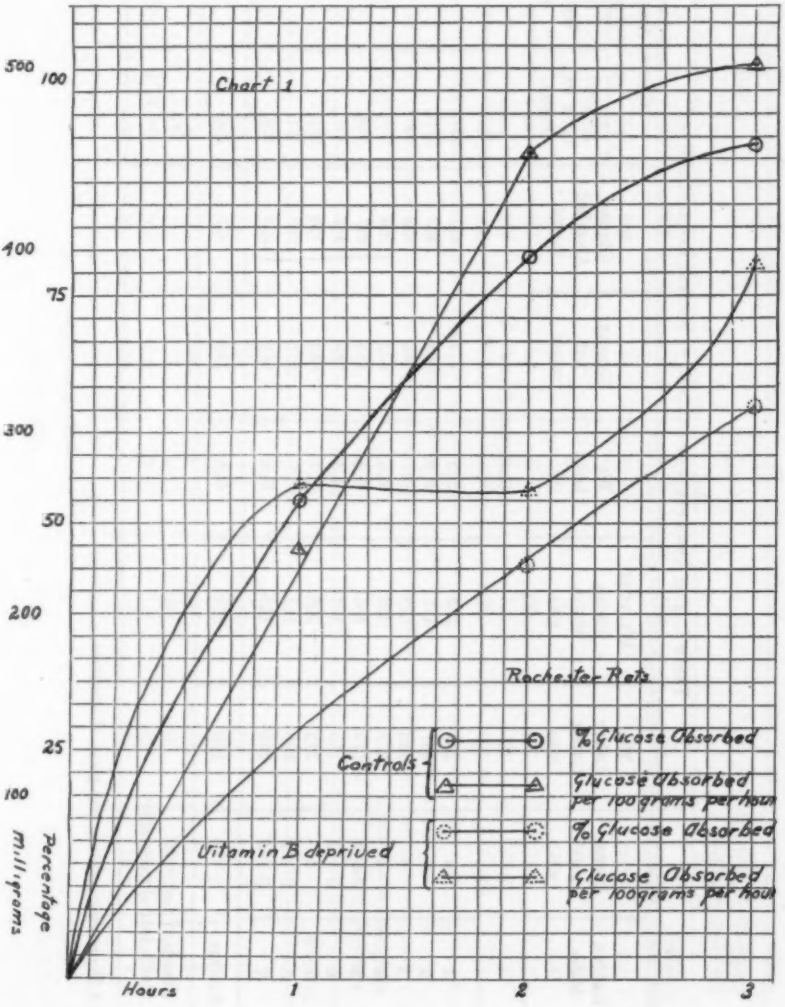

TABLE I. THE ABSORPTION OF GLUCOSE BY NORMAL ROCHESTER RATS[1]

Rat No. Males	Approx. Age mo.	Wt. before fasting gms.	Wt. after fasting 48 hrs. gms.	Wt. lost %	Glucose fed mgms.	Glucose Absorbed mgms.	Glucose Absorbed per hour per 100 gms. body wt. mgms.	Glucose Absorbed per hour per Sq. cm. body surface mgms.	Glucose Absorbed %
Absorption Period 1 hr.									
1	4	237	224	5.5	655	343	153	1.022	52.3
2	4	187	171	8.5	682	462	270	1.648	67.4
3	3	140	125	10.7	646	332	266	1.459	51.3
4	4	228	206	9.6	622	364	177	1.147	58.5
5	3-1/2	179	164	8.4	783	497	303	1.823	63.4
6	3-1/2	180	161	10.5	759	427	265	1.586	56.2
7	4	224	208	7.1	884	334	161	1.046	37.7
8	2-1/2	130	113	13.0	871	363	321	1.707	41.6
9	3-1/2	164	148	9.8	675	302	204	1.186	44.7
Average		185	169	8.2	731	381	236	1.40	52.6
Standard deviation							59.3	.289	
Coefficient of variability							25.13%	20.60%	

[1] 35 per cent solution was given throughout.

TABLE I *cont.*

Absorption Period 2 hrs.

1	3-1/2	173	150	13.2	880	718	203	1.398	81.5
2	3-1/2	190	171	10.0	880	686	201	1.224	77.9
3	3-1/2	159	140	11.9	895	757	271	1.542	84.5
4	3-1/2	162	140	13.5	895	731	261	1.489	81.6
5	3-1/2	161	144	9.6	885	673	234	1.346	76.0
6	3-1/2	183	167	8.7	882	694	208	1.257	78.6
7	3-1/2	166	149	10.0	890	677	227	1.324	75.9
Average		171	152	11.0	887	705	229	1.37	79.4
Standard deviation							26	.107	
Coefficient of variability							11.35%	7.82%	

Absorption Period 3 hrs.

1	3-1/2	170	153	10.0	814	778	170	.996	95.5
2	3-1/2	180	163	9.4	762	745	152	.914	97.7
3	3-1/2	161	150	6.8	878	842	187	1.092	95.8
4	3-1/2	173	151	12.7	895	868	192	1.121	96.9
5	3-1/2	168	147	12.5	895	697	158	.917	77.8
6	3-1/2	178	160	9.1	877	781	163	.971	89.0
7	3-1/2	191	173	9.4	881	803	155	.948	91.1
Average		174	157	10.0	857	788	168	.994	91.9
Standard deviation							14.6	.078	
Coefficient of variability							8.69%	7.85%	

Slightly over 50% of the glucose injected was absorbed in one hour, just under 80% for two hours and just over 90% for three hours. When the amount of sugar absorbed per 100 grams of body weight was plotted against time, a straight line was obtained by Cori but is not obtained in our results (see Chart 1). Practically speaking a straight line is obtained for the first two hours, but during the third hour there is a marked falling off in the absorption rate. An absorption of 52.6% of the glucose during the first hour, leaves 47.4% remaining in the alimentary tract. If 52.6% of the remaining 47.4% were absorbed during the second hour, 24.9% would be taken up, making the total amount absorbed 77.5% as against the figure of 79.4% which was found. If 52.6% of the remaining 22.5% were absorbed during the third hour, 11.8% would be taken up making the total absorption 89.3% in place of 91.9% which is found. It would appear from this agreement that the amount of glucose absorbed at all times is a function of the concentration of glucose remaining in the alimentary tract. Calculation of Cori's results on a similar basis reveals that the absorption for the first hour was 30.7%, leaving 69.3% unabsorbed. If the same percentage of the remaining 69.3% were absorbed the second hour, 21.3% would be taken up, making a total absorption of 52% as compared with 45.2% actually found. Of the remaining 48% a further absorption of 30.7% in the third hour would give 14.6%, making the total amount of glucose absorbed 66.6% as compared with 69.4%, the value actually found. Although the absorption was at a slower rate, there appears to be the same dependence upon the residual sugar as in our own results, upon the basis of percentage absorption. Variations are found in Cori's work which approximate those which we have obtained.

Table II contains the results obtained with rats from the Rochester colony which had been placed on a diet containing no vitamin B for periods of 41 to 46 days. Here again marked variations are to be noted among the individual total amounts of glucose absorbed and in the amounts absorbed per 100 grams of body weight per hour. The standard deviation based upon the absorption per 100 grams body weight for the one-hour period was 77.7 mgms. and the coefficient of variability 28.67% while the corresponding results calculated on the basis of surface area were 0.410 mgm. per sq. cm. and 27.78%. For the two-hour absorption period the standard deviation based upon 100 grams body weight was 29.1 mgms. and the coefficient of variability 21.72% while the values obtained on the basis of surface area were 0.182 mgms. per sq. cm. and 23.39%. Larger amounts of glucose were absorbed per 100 grams of

TABLE II. THE ABSORPTION OF GLUCOSE BY ROCHESTER RATS ON A VITAMIN B-FREE DIET[1] [2]

Rat No. Males	Weight time of placing on diet gms.	Weight Time Killed gms.	Weight Lost %	Glucose Fed mgms.	Glucose Absorbed mgms.	Glucose Absorbed mgms. per hour per 100 gms. body wt.	Glucose Absorbed mgms. per hour per sq. cm. body surface	Glucose Absorbed %
Absorption Period 1 hr.[3]								
1	255	142	44.3	1015	263	188	1.062	25.9
2	212	131	38.2	1209	257	196	1.095	21.2
3	190	119	37.3	1282	219	184	.995	17.1
4	186	103	44.6	1163	296	288	1.480	25.4
5	202	136	32.6	1287	433	319	1.799	33.6
6	188	114	39.3	1256	459	402	2.145	36.5
7	222	124	44.1	1255	397	320	1.759	31.6
Average	208	124	40.1	1209	332	271	1.476	27.3
Standard deviation						77.7	.410	
Coefficient of variability						28.67%	27.78%	
Absorption Period 2 hrs.[4]								
8	230	148	35.6	867	301	102	.569	34.7
9	243	154	36.6	870	502	163	.960	57.7
10	240	149	37.9	867	319	107	.623	36.7
11	260	157	39.7	956	507	162	.957	53.0
Average	243	152	37.5	890	407	134	.778	45.5
Standard deviation						29.1	.182	
Coefficient of variability						21.72%	23.39%	
Absorption Period 3 hrs.[4]								
12	228	137	39.9	871	535	130	.737	61.4
13	240	145	39.5	879	569	131	.755	64.7
Average	234	141	39.7	875	552	131	.746	63.1

[1] The approximate age of these rats at time of placing on diet was 4–1/2 months. [2] The rats were fasted for 24 hrs. only.
[3] 50 per cent solution given. [4] 35 per cent solution given.

body weight during the first hour than for normal rats. The reason for this is related to the higher percentage of glucose fed. See below.

The average weight of the rats after vitamin B deprivation for 41 to 46 days was 124 grams, although the original weights averaged higher than for the normal series. This smaller body weight would inevitably result in greater energy requirement per unit of weight, provided the vigor of the animals was kept up. It was of interest to see whether the absorption would be greater also per unit of body surface. Accordingly the absorption was calculated to milligrams per sq. cm. of body surface, using Meeh's formula ($S = kW^{2/3}$) in which the constant k has a value (Rubner (1902b)) of 9.1. Hill and Hill (1913) using results obtained with five rats, obtained an average value for k of 9.93, however we have used the value 9.1 for k in our calculations. The result indicates a closer relationship to body surface than to body weight during the one-hour absorption period (Tables I and II). There is a difference on this basis between the normal and vitamin B deprived animals of 5.71% while on the basis of body weight the difference is 15% during a one-hour absorption period. *If, however, the values obtained during the two-hour absorption period are used, there is a difference between the normal and vitamin B deprived animals on the basis of surface area of 76.09% while on the basis of body weight this difference is 55.97%.*

In starting the series on the vitamin B-free animals a change was made to a 50 per cent glucose solution with the thought that the greater concentration would reveal any defect of absorption all the more clearly, as indeed it does. But because such marked variations were being found, the percentage concentration of the glucose fed was reduced again during the two- and the three-hour absorption experiments to 35 per cent and the variations in the amounts of glucose absorbed were smaller. Notwithstanding the difference in concentration the percentages of glucose absorbed during the one- two- and three-hour periods all were materially less than those found for normal animals, as were the total weights of glucose absorbed.

The average results indicate a decreased glucose absorption during the second and third hours, there being a very marked decrease in the second hour, although the smaller amount fed leaves this conclusion a little in doubt. An absorption of 27.3% of the glucose during the first hour leaves 72.7% remaining. If 27.3% of the remaining 72.7% were absorbed the second hour, 19.8% would be taken up, making the total amount absorbed 47.1% as compared with 48.2% which was found. The same percentage of the remaining 52.9% would give 14.4% taken up, making the total absorption 61.5% in place of our result of 63.1%. It is evident also, from

the curves in Chart 1, that absorption cannot be entirely independent of concentration.

The rate of absorption of glucose in successive hours was not studied in the investigation at the Pennsylvania State College, all absorption periods being of one hour's duration and the strength of solution given always 35%. Table III contains the results obtained for the glucose absorption by normal rats, a few of which were litter mates, of the Penn State colony. These rats were older and larger than those used by Cori. The amount of glucose absorbed per hour per 100 grams of body weight varies within rather wide limits and there appears to be no relationship between the amount of glucose fed and the amount absorbed. The standard deviation per 100 grams body weight was 46.64 mgms. and the coefficient of variability was 33.55%. The standard deviation based on 1 sq. cm. surface area was 0.315 mgms. and the coefficient of variability 33.31%. It is likely that the absorption would be more nearly uniform if the amounts of sugar fed were more nearly proportional to the body weight. The average percentage of glucose absorbed by this group of animals was 33.5 for a one-hour period, while the average found by Cori under similar conditions was 31.8%. Both of these values are much lower than those found for rats of the Rochester colony, due possibly to a difference in the ages of the rats used. Variations were of the same order of magnitude as those found by Cori.

The results shown in Table IV are those obtained in the study of glucose absorption from the intestine of rats which had been on the diet used for the animals receiving no vitamin B, with the exception that 0.25 grams of yeast were fed daily to each rat. Because more than one animal had to be kept in a cage, the food intake is an average value. The food intake of these animals was greater than that of the animals receiving a diet totally deficient in vitamin B. This group of animals lost an average of 21% of their weight during the feeding period, while the animals having a diet totally deficient in vitamin B lost on the average 32% of their weight during a period of similar length. The amount of glucose fed was nearly uniform for these rats and yet the amount of sugar absorbed varied markedly. The standard deviation for glucose absorbed per 100 grams body weight was 43.10 mgms. and the coefficient of variability 28.92%. Using the figures for the absorption per square centimeter body surface the standard deviation was 0.259 mgms. and the coefficient of variability 27.63%. The percentage of glucose absorbed was somewhat less than that found for normal animals, but the absolute amount per 100 grams body weight is 149 milligrams as compared with 139 (average) for

TABLE III. THE ABSORPTION OF GLUCOSE BY NORMAL PENN STATE RATS

Rat No.	Approx. Age mo.	Wt. before fasting gms.	Wt. after fasting 48 hrs. gms.	Wt. lost %	Glucose fed mgms.	Glucose Absorbed mgms.	Glucose Absorbed mgms. per hour per		Glucose Absorbed %
							100 gms. body wt.	sq. cm. body surface	
1	6		293		998	305	104	.759	30.7
2	6		236		998	313	132	.906	31.3
3	6		249		678	213	85	.591	31.3
4	6		239		681	221	92	.631	32.6
5	6		213		681	172	81	.530	25.3
6	12		300		1179	417	138	1.046	35.4
7	6		181		1164	457	218	1.570	39.2
8	7	206	199	3.4	1010	369	185	1.187	36.5
9	7	213	203	6.6	932	390	196	1.254	41.8
10	7	218	194	6.9	919	374	185	1.190	40.7
11	7	205		4.2	932	224	115	.735	24.0
Average		211	228	5.2	925	314	139	.945	33.5
Standard deviation							46.64	.315	
Coefficient of variability							33.55%	33.31%	

TABLE IV. THE ABSORPTION OF GLUCOSE BY PENN STATE RATS ON A VITAMIN B-DEFICIENT DIET PLUS 0.25 GM. YEAST per DAY

Rat No. %	Wt. Time Placing on Diet gms.	Weight When Killed gms.	Weight Lost %	Glucose fed mgms.	Glucose Absorbed mgms.	Glucose Absorbed mgms. per hour per		Glucose Absorbed %	Average Daily Food Intake gms.
						100 gms. body wt.	sq. cm. body surface		
1	245	178	27.3	1008	366	206	1.208	36.3	7.0
2	233	162	30.4	992	322	199	1.191	32.5	5.8
3	272	208	23.5	978	222	107	.695	22.7	6.3
4	268	209	22.0	970	225	107	.702	23.1	6.3
5	259	203	21.6	1022	383	189	1.217	37.4	7.8
6	261	222	14.9	1045	425	191	1.274	40.6	8.0
7	228	179	21.4	974	254	142	.879	26.0	7.0
8	276	194	29.7	962	151	78	.495	15.9	7.0
9	255	213	16.4	955	336	158	1.035	35.1	8.0
10	249	200	19.6	981	201	100	.646	20.4	6.6
11	202	149	26.2	986	244	164	.954	24.7	5.8
Average	250	192	21.4	988	284	149	.936	28.6	6.8
Standard deviation						43.10	.259		
Coefficient of variability						28.92%	27.63%		

TABLE V. THE ABSORPTION OF GLUCOSE BY PENN STATE RATS ON A VITAMIN B-FREE DIET

Rat No.	Wt. Time Placing on Diet gms.	Weight When Killed gms.	Weight Lost %	Glucose fed mgms.	Glucose Absorbed mgms.	Glucose Absorbed mgms. per hour per		Glucose Absorbed %	Average Daily Food Intake gms.
						100 gms. body wt.	sq. cm. body surface		
1	195	165	15.4	587	297	180	1.085	50.5	
2	160	126	21.2	593	182	144	.796	30.6	
3	263	174	33.8	697	362	208	1.276	51.8	4.9
4	223	142	36.3	703	305	215	1.595	43.4	4.9
5	265	184	30.6	701	175	95	.594	25.5	4.9
6	250	165	34.0	703	325	197	1.187	46.2	4.7
7	291	207	28.5	698	181	88	.568	25.8	5.8
8	309	207	33.0	698	224	109	.703	31.7	5.8
9	317	237	25.2	696	258	109	.740	37.0	5.8
10	260	161	38.1	977	217	135	.806	22.2	4.8
11	260	173	33.5	963	180	104	.637	18.5	4.8
12	305	215	29.5	975	300	140	.919	31.8	
13	294	159	45.9	1056	344	216	1.288	32.5	
14	342	187	45.3	1041	326	174	1.095	31.3	6.9
Average	248	179	32.2	792	269	151	.949	34.1	5.33
Standard deviation						44.87	.299		
Coefficient of variability						29.72%	31.52%		

the normals. The results obtained with normal Penn State rats which had received a diet deficient in vitamin B for 33 to 39 days are given in Table V. The amount of glucose fed was not constant, but, as noted before, there was no relationship between the amount fed and the amount absorbed. Neither body weight nor loss in body weight appear to have any effect upon the amount of glucose absorbed. The rats in this group had lost an average of 32% of their body weight, while the rats obtained from the Albino Supply Company had lost 39% and the Rochester rats 40% of their body weight under similar conditions. The amount of glucose absorbed by the vitamin B deprived animals was less than that absorbed by the normal rats.

The absorption per 100 grams body weight per hour was slightly less with the normals than with the animals receiving the deficient diet; however the difference in these figures is less than 10% and the significance of this is to be questioned. Although the percentage absorption of glucose is somewhat less than normal in those animals which had been on a diet partially deficient in vitamin B, the percentage of absorption in case of the animals on a diet totally deficient in vitamin B is slightly greater than that of normals. This observation is of particular interest in light of the fact that the Penn State rats have been able to resist the effects of vitamin B deprivation. The other two groups of animals did show a decrease in the amount of glucose absorbed. The standard deviation calculated on the basis of absorption per 100 grams body weight was 44.87 mgms. and the coefficient of variability 29.72% while the standard deviation for the results based upon one sq. cm. surface area was 0.299 mgms. and the coefficient of variability 31.52%.

Table VI shows the values obtained for glucose absorption with normal animals purchased from the Albino Supply Company. The amounts of glucose fed were comparatively uniform and the amounts absorbed were also more nearly uniform. The standard deviation based upon absorption per 100 grams body weight was 17.06 mgms. and the coefficient of variability 12.83%, while for absorption per square centimeter body surface the standard deviation was 0.111 and the coefficient of variability 14.87%. Body weight does not appear to influence the total amount of glucose taken up. However, the heavier rats tend to absorb less glucose per 100 grams of body weight than do the lighter animals. The amount of glucose absorbed per 100 grams of body weight per hour is considerably less than the amount observed by Cori. A difference in age of between four and eight months had little effect upon glucose absorption. The percentage of absorption is slightly less with these rats than with the Penn State

TABLE VI. THE ABSORPTION OF GLUCOSE BY NORMAL ALBINO RATS ON AN ADEQUATE DIET

Rat No.	Approx. Age mo.	Wt. before fasting gms.	Wt. after fasting 24 hrs. gms.	Weight lost %	Glucose fed mgms.	Glucose Absorbed mgms.	Glucose Absorbed mgms. per hour per		Glucose Absorbed %
							100 gms. body wt.	sq. cm. body surface	
1	8	276	259	6.15	1033	333	128	.901	33.2
2	8	266	252	5.26	1006	295	117	.813	29.4
3	8	224	212	5.35	1012	230	109	.711	20.2
4	4	315	296	6.03	985	318	108	.787	32.3
5	4	327	304	7.03	952	257	84	.625	26.9
6	4	326	304	6.74	942	267	88	.649	28.2
7	4	285	270	5.26	944	334	124	.879	35.4
8	8	254	246	3.15	973	283	115	.792	29.9
9	4	335	322	3.88	973	250	78	.587	25.9
Average		290	274	5.42	980	285	106	.749	29.04
Standard deviation							17.06	.111	
Coefficient of variability							16.10%	14.87%	

normals, and materially less than with normals from the Rochester colony.

The results obtained with the rats that came from the Albino Supply Company, which had been on a diet partially deficient in vitamin B, are shown in Table VII. The amount of glucose fed to this group of animals has been fairly uniform. These rats had lost on an average 25% of their original body weight during the period of the diet as compared with a 21% loss found with the Penn State rats. The food intake of these rats was greater than with the corresponding group on a diet totally deficient in vitamin B and somewhat higher than in the case of the Penn State rats on the same diet. The amount of glucose absorbed varied within wide limits. The standard deviation calculated on the basis of absorption per 100 grams of body weight was 30.6 mgms. and the coefficient of variability 23.91%, while the corresponding figures for absorption per square centimeter body surface were 0.196 mgms. and 23.42%. There is no apparent correlation between the body weights of the rats and the total amounts of glucose absorbed. The amount of glucose absorbed is slightly less than that observed for the normals of this group. The percentage of glucose absorbed is a fraction of a per cent higher than that of the control group.

Table VIII contains the results obtained with Albino rats which had been on a diet totally deficient in vitamin B for a period of from 39 to 45 days. The amounts of glucose fed were nearly uniform and closely approximate the amounts fed to the corresponding group of rats on a diet partially deficient in vitamin B. These rats had lost an average of 39% of their body weight during the feeding period as compared with an average loss of 32% found for the Penn State rats, and 40% for the Rochester rats under similar conditions. The amounts of glucose absorbed were not at all constant nor were the amounts absorbed per 100 grams body weight per hour. The standard deviation based upon the absorption per 100 grams body weight was 34.1 mgms. and the coefficient of variability 33.46%, while for absorption per square centimeter body surface the standard deviation was 0.204 mgms. and the coefficient of variability 32.48%. The total amount of glucose absorbed was materially less than with the normal animals and those on a vitamin B-deficient diet plus yeast. The absorption per 100 grams body weight was slightly less than with the control rats. However, the percentage of glucose absorbed was about one-third less. The average of 20% absorption is the lowest value found for any of the rats on experimental diets. There was no correlation between body weight and the total amount of glucose absorbed. The food intake of the individual animals has had no apparent effect upon the amount of absorption,

TABLE VII. THE ABSORPTION OF GLUCOSE BY ALBINO RATS ON A VITAMIN B-DEFICIENT DIET, PLUS 0.25 GM. YEAST PER DAY

Rat No.	Wt. Time of Placing on Diet gms.	Wt. When Killed gms.	Wt. Lost %	Glucose fed mgms.	Glucose Absorbed mgms.	Glucose Absorbed mgms. per hour per		Glucose Absorbed %
						100 gms. body wt.	sq. cm. body surface	
1	288	217	24.6	792	235	108	.715	29.7
2	302	235	22.1	792	180	77	.519	22.8
3	278	218	21.5	780	195	90	.592	25.0
4	289	220	24.0	844	280	127	.844	33.1
5	287	218	24.0	923	282	129	.856	30.5
6	259	203	21.6	915	257	127	.818	28.1
7	294	232	21.0	891	189	82	.550	21.3
8	302	230	23.8	990	288	125	.843	29.3
9	299	211	29.4	981	327	155	1.014	33.3
10	273	198	27.4	959	302	153	.977	31.5
11	285	180	36.8	956	249	138	.858	26.1
12	290	200	31.0	970	356	178	1.144	35.6
13	301	233	22.5	964	394	169	1.143	40.8
Average	288	215	25.3	904	273	128	.836	29.7
Standard deviation						30.6	.196	
Coefficient of variability						23.91%	23.42%	

TABLE VIII. THE ABSORPTION OF GLUCOSE BY ALBINO RATS ON A VITAMIN B-FREE DIET

Rat No. Males	Wt. Time of Placing on Diet gms.	Weight When Killed gms.	Weight Lost %	Glucose fed mgms.	Glucose Absorbed mgms.	Glucose Absorbed mgms. per hour per		Glucose Absorbed %	Average Daily Food Intake gms.
						100 gms. body wt.	sq. cm. body surface		
1	313	202	35.4	828	236	117	.753	28.4	6.6
2	276	166	39.8	897	167	101	.608	18.6	6.4
3	306	186	39.2	891	192	103	.648	21.4	5.6
4	292	185	36.6	897	213	115	.721	23.8	5.6
5	294	178	39.4	1006	167	94	.580	16.6	5.9
6	277	149	46.2	974	112	75	.438	11.4	7.5
7	300	179	40.3	897	225	125	.778	25.0	6.2
8	292	166	43.1	897	218	131	.793	24.3	6.0
9	329	205	37.6	897	180	88	.569	20.0	6.0
10	303	181	40.2	897	60	33	.206	7.0	6.0
11	298	179	39.9	990	277	155	.958	27.8	6.0
12	277	150	45.8	944	223	149	.868	23.6	5.2
13	301	195	35.2	799	94	48	.307	11.9	7.0
14	270	165	38.8	830	213	129	.778	25.6	7.0
15	305	198	35.0	830	130	66	.421	15.7	7.0
Average	296	186	39.2	898	181	102	.628	20.0	6.26
Standard deviation						34.1	.204		
Coefficient of variability						33.46%	32.48%		

although it should be mentioned that group feeding was used because of lack of available space, and two or three rats were kept in a cage.

Discussion of Results

Mattill (1923) observed that rats deprived of vitamin B which have received either glucose or sucrose show a rise in the respiratory quotient, although this rise is less than in the case of normals. He has suggested that the reason for this may lie in a delayed absorption. Our results tend to show that this may be true (See Chart 1). Miller and his coworkers (1920) have observed, however, with human subjects, that feeding large amounts of glucose or sucrose in concentrated solution depressed gastric secretion and delayed evacuation of the stomach. Calculated on the basis of grams of sugar per kilogram of body weight the amount of sugar used for humans by Miller and the amount used by us for rats is approximately the same.

Experiments of this kind may be vitiated by the fact that refusal of food accompanies vitamin B deprivation as Mitchell (1927) (1928) has pointed out. Luchetti (1918) has found that absorption from the intestine is slower in dogs which have fasted than in normal dogs. It is extremely difficult to distinguish between the effects of vitamin B deprivation and inanition.

The question may be raised as to possible explanations for the difference in behavior between the Penn State rats and the rats of the other two groups. Young rats of the Penn State colony show little or no effect of vitamin B deprivation over a period of a year other than a failure to grow. Older rats of this same colony when placed on a diet containing no vitamin B lose weight slowly and their appetite diminishes. It should be remembered that the food intake of the Penn State rats remained at a higher level during vitamin B deprivation than that noted for rats obtained from the Albino Supply Company. The Penn State rats did not lose as much weight as rats of the other groups under the same conditions. The Penn State rats were pied, and the other groups were albino, so that racial differences may have affected the results. With rats of the other two groups there was a marked decrease in the absorption of glucose when vitamin B was absent from the diet. We cannot account for the lowered absorption with the Penn State rats that had been receiving yeast with the partially B-deficient diet. Additional observations might have shown this difference to be of a lesser value.

The variations in the percentage glucose absorbed with the Penn State rats on a vitamin B-free diet were much greater than those noted with the

two other groups which makes the interpretation of these results difficult. With the Penn State rats it would appear that vitamin B deprivation had variable effects. The differences noted with animals of different origin illustrate the necessity of using a standard group of animals for such investigations. The experimental work with the Rochester rats was performed during the winter months, while the work with the other two groups of rats was done during the spring and summer. That the time of year during which the observations were made may account for some of the differences noted between the Rochester rats and the other groups is to be doubted because all were subjected to about the same temperature, although Riddle and Honeywell (1924) have found that cold weather causes a marked lowering in the concentration of blood sugar in pigeons kept outdoors.

Mottram, Cramer and Drew (1922) have shown that the presence of vitamins in the food, particularly vitamin B, causes a more rapid absorption of fat from the intestine. Never (1928), after having studied the effect of vitamin-free diets upon the secretion and motility of the intestinal tract, and upon appetite, concludes that these three are disturbed secondarily and the most significant disturbance lies in absorption from the intestinal tract.

Seth and Luck (1925) studied the absorption of amino acids from the intestinal tract by analyzing the blood of the animals used (rabbits and dogs), determining the amino acid and urea content. In general there was a rapid rise in the amino acid nitrogen in rabbit's blood during the first two hours, following the ingestion of amino acids, after which there was a smaller increase or a decrease. In a subsequent experiment, in which dogs were used, the amino acids were injected into the small intestine by means of a hypodermic needle and a pipette. The amino nitrogen in the arterial blood tended to increase rapidly after the injection, and gradually fell off, the same holding true for the amino nitrogen in the portal blood. The urea content of the blood increased more rapidly early in the period and tended to decrease after one or two hours. The results indicate that absorption does not take place at a uniform rate, for if it did, we should expect to find a consistent and uniform increase in the amount of amino nitrogen in the portal blood.

The statistical treatment of the results obtained is of questionable value, because of the small numbers of animals used in some of the groups, except to emphasize the large variability of the results.

The authors plan to continue this investigation.

SUMMARY

1. The method devised by Cori for the quantitative study of the absorption of glucose by the whole alimentary tract has been used.

2. Three different groups of animals were used, one group from the Rochester colony, a second from the Penn State colony and a third from the Albino Supply Company. The Penn State rats were pied, while the other two groups were albino. The results obtained with the different groups do not correspond closely.

3. The rate of absorption of glucose was not uniform for the one- two- and three-hour periods, this being particularly noticeable in case of the rats which had received a diet deficient in vitamin B.

4. The total amount of glucose absorbed and the amount absorbed per 100 grams of body weight per hour by normal and vitamin B-deprived animals varied within wide limits. These variations were not dependent upon body weight nor upon the actual amount of glucose fed.

5. Absorption per square centimeter of surface area varies markedly, although the results indicate that there is a slightly closer relationship between absorption and body surface than between absorption and body weight.

6. The percentage of glucose absorbed during one-, two- and three-hour periods appeared to be dependent upon the amount of glucose remaining unabsorbed in the alimentary tract.

7. The Rochester rats which had been on a diet containing no vitamin B absorbed a smaller percentage of the glucose fed than did normal animals.

8. Vitamin B deprivation had little effect upon absorption in rats of the Penn State colony, rats on a diet totally deficient in vitamin B absorbing practically the same amount of glucose as the normal animals. Rats which had been on a B-deficient diet but had received yeast did absorb a lesser percentage of the glucose fed.

9. Rats from the Albino Supply Company, which had received a diet containing no vitamin B absorbed a smaller amount of the glucose fed than did normal animals. Rats on the same B-free diet which had received yeast showed little difference in absorption as compared with normals.

10. The percentage of glucose absorbed by normal animals of the Penn State colony and the Albino Supply Company closely approximates that found by Cori.

11. The variation in the percentage of absorption with our normal animals is somewhat greater than the percentage variation to be noted between the absorption coefficients found by Cori.

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Editorial Review

THE PHYSIOLOGICAL EFFECTS OF PROTEIN

ASIDE from their value in furnishing energy to the animal organism and matter for the construction of its tissues and their secretions, the food nutrients may modify tissue functioning during the period of their transportation to the tissues and of their metabolism. The specific dynamic effect of the organic nutrients is an outstanding example of such a modification of tissue functioning.

The effects of certain vegetable oils on the intestinal tract and the profound effect of sugar ingestion in certain cases of pathological hypoglycemia are other instances of the same category. The physiological effects of proteins, however, are more varied than those of other nutrients and may be so potent as to render advisable a restriction of the protein intake under certain conditions and in certain diseases. The great practical importance of an inquiry into the physiological effects of protein is thus apparent.

The calorigenic effect of ingested protein is its most marked physiological effect. Compared with the effect of other nutrients, that of protein is much more pronounced not only with regard to the total excess heat produced, but also with regard to the peak effect and the period of excess heat production, the basis of comparison being 100 calories of nutrient. The most comprehensive study of this specific dynamic action of the organic nutrients is that of Lusk and his coworkers. This study has indicated clearly that, for pure nutrients, the specific dynamic action is not due appreciably to the events occurring within the intestinal tract, as von Mering and Zuntz believed, nor to the intermediary reactions of metabolism, as Rubner assumed, but that it is due to the direct stimulatory effect of the end-products of digestion upon the cells themselves. The stimulus may be due merely to a plethora of oxidizable nutrients within or around the cells, in which case the nutrient affording the stimulus is itself oxidized, or it may be the result of a direct interaction between compounds of a definite structure and the tissue cells, in which case the increase in heat production may involve other nutrients than the one affording the stimulus. Lusk's investigations led him to believe that the specific dynamic action of fats and sugars was a stimulus of the former type, i.e., a metabolism of plethora, but that the specific dynamic action

of proteins and amino acids was a stimulus of the second type, less readily characterized.

Throughout Lusk's investigations, attempts to explain the specific dynamic effects of amino acids are complicated by the difficulty of analyzing the extra heat production in terms of carbohydrate calories, fat calories and amino-acid calories during a period of greatly variable amino-acid catabolism. This difficulty resides in the fact that there is no generally accepted criterion of the rate of amino-acid catabolism or of protein catabolism, so that results obtained by the use of any particular criterion cannot be accorded any great degree of finality.

However, some evidence has been secured by Lusk that the amino-acid stimulus to heat production is qualitatively as well as quantitatively different from the metabolism of plethora due to the inflow of sugar molecules from the intestinal tract. Thus, the ingestion of 70 grams of glucose by a phlorhizinized dog was found (1) not to increase the heat production, although in the normal dog an increase of 35 per cent over the basal value was obtained in the second, third, and fourth hours after its administration. Evidently a metabolism of plethora does not supervene if the organism is unable to utilize the sugar by reason of phlorhizin poisoning. However, an ingestion of 12.5 to 20 grams of glycine or of alanine produced a marked rise in the metabolism of the phlorhizinized dog, although no oxidation of the amino acids could have occurred, since they were quantitatively excreted in the urine as glucose and urea. Apparently the amino acid stimulus is not due only to a plethora of oxidizable molecules in the tissues.

Another indication of a qualitative difference between the specific dynamic effect of glucose and of amino acid was obtained by Anderson and Lusk (2) in experiments on dogs at rest and at work on a treadmill. The results reported show that when muscular work is being performed during the hours following a large ingestion of glucose, the metabolism rises to about the same height as when the same intensity of work is being done during a period when the gastro-intestinal tract is free from food. On the other hand, after the ingestion of meat or of alanine there is a most exact summation of the increment in heat production due to specific dynamic action and that due to muscular activity. Evidently when glucose is used in metabolism as rapidly as it is transported from the intestinal tract, no plethora of oxidizable material accumulates in the tissue fluids and consequently no stimulus to cellular activity results. With amino acids, however, the characteristic stimulus to metabolism appears to be quite independent of variations in energy requirements and, hence also, of a

plethora of oxidizable material. The argument may, however, need revision if it develops from the work of Hill and Meyerhof and their associates on the source of muscular energy that glucose is directly available for muscular work, while amino acids are not.

If the amino acid stimulus to heat production is not merely a metabolism of plethora, it would appear to be related to a specific reaction between the tissues and some chemical structures or groupings found in amino acids or in their normal metabolites. This view is strengthened by the fact that different amino acids differ greatly in their specific dynamic effects. Glycine and alanine were early shown to have a powerful effect, whereas leucine and tyrosine produced only an inconsiderable effect, and glutamic acid no effect whatever. Later Atkinson and Lusk (3) demonstrated the inactivity of aspartic acid, the other dibasic amino acid present in the protein molecule, while in 1927 Rapport and Beard (4) observed a greater specific dynamic effect for phenylalanine than that usually obtained for glycine, an effect for tyrosine comparable to that for alanine, and no calorigenic activity for valine whatever. Wilhelmj and Bollman (5) have confirmed the great heating effect of alanine, glycine, and phenylalanine following intravenous, instead of oral administration. It is a curious anomaly, however, the explanation of which is still under investigation, that the specific dynamic effects of proteins do not appear to be related to their amino acid constitution (6).

In his earlier publications up to April of 1915, Lusk was inclined to ascribe to the unchanged amino acids the cellular stimulation following protein or amino acid ingestion. Later, however, he changed his views in favor of the keto or hydroxy acids formed by the deamination of the amino acids. In this connection, an early experiment by Rubner (7) was cited, indicating that, when ingested protein is retained in the body in growth, it exerts no specific dynamic effect. An experiment of Hoobler (8) on an infant was given the same significance. However, it may be pointed out that these experimental data, on critical study, appear to be quite inadequate to establish such an important distinction between the physiological effects of amino acids undergoing anabolism and those undergoing catabolism.

A series of experiments were then undertaken by Lusk and his associates (9) to decide whether the non-nitrogenous products of the deamination of glycine and alanine are not entirely responsible for their heating effects on ingestion by animals. The data obtained, however, were conflicting and difficult to reconcile with his revised opinions, and in his last paper on this question Lusk says: "While one may still speak of amino-

acid stimulation in the sense that one speaks of the specific dynamic action of protein, one is still far removed from an actual elucidation of what transpires in the cells to induce the phenomenon."

On the other hand, considerable may be said for the view that the amino acids themselves are the cell stimuli involved in specific dynamic action. The catalytic action of amino acids with reference to certain chemical reactions has been well established. Ort and Bollman (9a) demonstrated that cystine, glycine, alanine, phenylalanine, leucine, histidine and valine catalyze the oxidation of glucose by hydrogen peroxide, while glutamic acid, aspartic acid, and tyrosine do not, while Witzemann (9b) found that glycine and certain glycolates catalyze the oxidation of butyric acid with H_2O_2 to a marked extent. Of particular significance to this question is the work of Rapport and Katz (9c) demonstrating that glycine exerts its characteristic calorigenic effect when perfused through an isolated group of muscles. Therefore, the specific dynamic effect of glycine is exerted upon the muscle cells directly, without the intervention of any other tissue or organ. On the basis of the experiments of Bollman, Mann and Magath (9d), indicating clearly that deamination is a function peculiar to the liver, the results of Rapport and Katz permit of no other interpretation than that glycine itself is a powerful stimulant to the metabolism of quiescent muscle cells.

Another phase of the specific dynamic action of protein relates to the effect of the nervous system, the endocrine glands, and certain seemingly hereditary factors involved in the production of obesity. Aub, Everett, and Fine (10) observed no increase in heat production when glycine was injected intravenously into cats anesthetized by urethane or paraldehyde. On the other hand, the intravenous injection of glycine, but not of glutamic acid, produced a definite specific dynamic effect in the decerebrate cat. Similarly Liebeschütz-Plaut and Schadow (11) report that the calorigenic effect of protein induced by oral administration continues after all the nerves to the small intestine are severed. The severing of the splanchnic nerves, according to Asher and Nayama (12), increases the specific dynamic effect of meat as well as sugar.

The investigation of human subjects suffering from various pathological disorders, involving the nervous system or the endocrine glands, are suggestive of a number of endogenous factors that may influence the effect of ingestion of protein on heat production. Those of Plaut (13), Rolly (14), Bernhardt (15), and Liebesny (16) indicate that hypophyseal (pituitary) disorders are frequently associated with diminished specific dynamic action of protein. Liebesny's experiments also show that a pathological

condition of the autonomic nervous system, as in certain neuroses, Raynaud's disease, and scleroderma, may have the same effect.

The evidence with reference to the relation of the thyroid to the specific dynamic action of protein is mainly negative. Investigations of patients with Graves' disease and myxedema, in which the thyroid is over- or underfunctioning, respectively, indicate a specific dynamic action of food or of protein that is within normal limits (13, 14, 16, 17, and 18). However, Abelin (19) in respiration experiments with rats, obtained a greater specific dynamic effect of protein after *per os* administration of thyroid material, although contrary evidence may be found in the literature. The evidence with respect to other glands, i.e., the spleen and the gonads, is also conflicting or inconclusive.

Wang and Strouse (20), in respiration experiments on 12 obese subjects, found markedly subnormal specific dynamic effects of proteins, carbohydrates, and fats. No clinical description of these subjects is given and the results obtained were not referred to any particular glandular deficiency. Similar results are reported by Jaquet and Svenson (21), though in the absence of normal standards of comparison, their significance is not so evident. Gibbons (22) observed a greater specific dynamic effect of meat in a thin dog (a thorough-bred greyhound) chosen as having a hereditary tendency to leanness, than in a stocky mongrel (chiefly St. Bernard or mastiff), selected as an animal tending toward adiposity by racial inheritance.

Two plausible explanations of these endogenous factors that modify the specific dynamic effect of protein suggest themselves, i.e., (a) either these factors modify the rate at which the amino acids resulting from the digestion of protein reach the tissues, and hence the effective concentration of the stimulant, or else (b) they modify the irritability of the tissues with respect to the amino acid stimulus. Liebeschütz-Plaut and Schadow (23) have presented evidence to the effect that in 10 cases of pathologically reduced specific dynamic action of protein an abnormally low aminoacidemia occurred after the ingestion of protein.

Besides its immediate effect on the heat production of the animal organism, the ingestion of protein continuously, exerts a marked effect upon the post-absorptive metabolism somewhat proportional to the plane of protein nutrition. This residual calorigenic effect of protein, sometimes referred to as the "secondary specific dynamic effect," has been related to the amount of so-called "deposit protein" in the tissues, the very flexible reservoir of nitrogenous material that is increased with each increase in protein intake, and that is slowly depleted without detriment to the animal

on lowering the level of protein nutrition. The proof of the existence of this unstable deposit of nitrogen in the tissues rests entirely upon the results of nitrogen balance studies. While it is commonly called a storage of protein, the fact that the excessive feeding of zein, the incomplete protein of corn, also leads to a prolonged lag in the adjustment of the urinary nitrogen to the endogenous level in a subsequent period of starch feeding (24), might be taken to indicate that the temporary retention of nitrogen on high protein levels is not necessarily the result of a retention of protein.

However this may be, it was shown by Rubner (7, p. 246) that the ingestion of excessive amounts of protein by a dog for several days occasions a gradual rise in total heat production until nitrogen equilibrium is attained. Similarly, Atkinson, Rapport, and Lusk (25) noted that the basal metabolism of a dog on a mixed diet increased 23 percent after 8 days of meat feeding and that, after a return to a mixed diet, the higher basal metabolism persisted for more than two and one-half weeks. Benedict and his coworkers (26) were the first to associate the loss of deposit protein—called by them "surplus cellular nitrogen"[1]—with the reduction in the basal metabolism of men consequent upon a marked reduction in caloric intake. The recent contribution of Deuel, Sandiford, Sandiford, and Boothby on the nitrogen minimum in man (27) includes a very complete study of the relation between the loss of deposit protein and the basal metabolic rate. They observed a rapid fall in the basal heat production during the first 8 days of protein-free feeding coincident with a rapid fall in the elimination of nitrogen by the kidneys. Thereafter, when the nitrogen excretion fell to lower levels, the basal metabolism remained practically constant. Subsequently, when the subject was put upon a high protein diet and was replenishing his stores of deposit protein, the basal metabolism was definitely elevated, even above the previous level.

If the basal metabolism of an individual is related to his vitality and physiological efficiency, an interpretation for which there is much to be said, then the relation of the dietary protein to this important factor of health and well-being is clear. On the other hand, the immediate heating effect of protein will modify its dietetic value in accordance with external conditions. In the colder climates, and in the colder seasons in temperate climates, a relatively high protein intake is of distinct advantage to comfort, while under warm environmental conditions the reverse is true. The high-protein diets prevailing in the arctic regions and the low-protein

[1] In some respects it would seem that this term is a better one than "deposit protein." The latter is used in this discussion because of its more general currency.

vegetarian diets prevailing in the tropics testify to the truth of this generality.

The significance of deposit protein has a bearing on the question of optimum protein nutrition. In the opinion of many, animals thrive better on amounts of protein above the minimum requirements. The conception of optimum nutrition implies that there is an optimum intake of protein greater than the minimum capable of covering all the requirements of protein for animal functioning, which in turn implies that the mere presence of an excess of protein (or amino acids) in the body tissues and fluids exerts a favorable physiological effect. The facts concerning the significance of deposit protein that have just been considered are not inconsistent with this belief, though its direct experimental justification is difficult.

The theory of optimum protein nutrition has been applied particularly to the dairy cow. Although early investigations in this country and in Europe may be cited in support of the view that an excess of protein above the requirements of maintenance and the production of milk stimulates milk secretion, even up to very high levels of protein intake, and although current practice in feeding cows on test is based upon the verity of this proposition, more recent investigations have not afforded any considerable support for it. Besides establishing the fact that the protein requirements for milk production are much lower than the current feeding standards would indicate, the experiments of Haecker (28), Hills and associates (29), Ellett, Holdaway and Harris (30), Fries, Braman, and Kriss (31), Perkins (32), and particularly Buschmann (33) indicate clearly that little if any increase in milk production resulted from increasing the protein intake above the minimum requirements. Nevens' self-feeding experiments on dairy cows (34) also offer no support—in fact, quite the contrary—for the belief that nitrogenous concentrates are stimulants to the mammary glands. These negative results cannot, of course, dispose of the positive results obtained in other experiments, but in demonstrating that excess protein does not *always* stimulate the mammary glands, they cast some doubt on the significance of increases in milk production obtained in other experiments subsequent to increases in the intake of high protein feeds. It becomes a debatable question whether the increased activity of the mammary glands was the result of an increased protein stimulus, or an increased stimulus produced by other feed constituents simultaneously increased in the ration.

The possibly deleterious physiological effects of the ingestion of amounts of protein above the minimum requirements of animals, particularly of

man, has occasioned a large amount of discussion and of experimental investigation. The question at issue is whether proteins are nutrients that should be partaken of sparingly, because their digestion and metabolism exposes the cells to the action of metabolites that may disturb their normal functioning, and imposes a burden upon the kidneys in eliminating them from the body.

Probably the first investigation apparently affording experimental verification for the belief that protein is capable of exerting such deleterious effects, was that of Chittenden (35). This investigation involved a large number of observations upon three groups of human subjects subsisting upon low-protein diets for several consecutive months. It was found that a marked reduction in the allowance of protein prescribed by current dietary standards not only failed to induce malnutrition, but actually appeared to promote the health and well-being of the subjects. Chittenden, on the basis of these results, eulogized the low-protein régime, and enunciated a doctrine of physiological economy in nutrition that has found its way, in one form or another, into several systems of dietetics. The later studies of Hindhede (36) have established the adequacy, in adult nutrition at least, of protein intakes even smaller than those investigated by Chittenden.

However, in the now classical experiments of Chittenden the absence of control groups of subjects maintained upon the usual protein intake but otherwise subjected to the same routine of life as the other groups, must be keenly felt by the critical apparaiser of their significance, and renders questionable the deduction that the observed benefits of the experimental conditions imposed were due solely, if at all, to the reduction in protein intake. There are communities and races of men that have subsisted for generations on small amounts of protein comparable to those advocated by Chittenden and Hindhede, but their physical, intellectual and industrial characteristics are not such as to inspire confidence in the wisdom of their habits or any desire to follow in their footsteps. Among such communities, the low-protein diets in vogue are not a matter of choice but a matter of necessity, due to a food supply restricted by climate, geographical location, or industrial backwardness.

It has seemed a reasonable proposition to many physiologists that the elimination of the nitrogenous metabolites of protein should constitute an onerous burden on the kidneys, and that the continuous ingestion of immoderate amounts of protein would ultimately prove harmful to these essential organs. In apparent confirmation of this proposition, Newburgh in 1919 (37) reported experimental observations on rabbits fed high-

protein diets. Acute, subacute or chronic nephritis was noted in all cases and was attributed to the excessive protein intake. Again in 1923, Newburgh and Clarkson (38) described dilatation of the urinary tubules and some scarring of the glomeruli in the kidneys of rabbits subsisting for 6 to 12 months on diets containing 27 to 36 percent of protein. Squier and Newburgh (39), in experiments on human subjects, observed the appearance of red blood corpuscles in the urine and the production of albuminuria or the aggravation of an existing albuminuria in patients with varying degrees of kidney damage following forced high-protein feeding. Furthermore, forced high-protein feeding with normal men invariably induced the appearance of red blood corpuscles in the urine.

In an attempt to explain the renal injuries apparently induced by high-protein feeding, Newburgh and Marsh (40) determined the effects on the kidneys of rabbits and puppies of the intravenous injection of individual amino acids. The intravenous administration of alanine, leucine, glycine, phenylalanine, and glutamic acid gave no evidence of renal injury in doses as large as 2 grams per kgm. of body weight. Arginine and aspartic acid were midly nephrotoxic, while serious kidney damage was produced by lysine, histidine, tyrosine, tryptophane and cystine. Evidence of the occurrence of kidney injury was obtained by microscopic examination of the urine and of the kidney itself. Urea was without effect upon the kidney. Lewis (41) has reported the production of serious renal injury in rabbits by the oral administration of the sodium salt of cystine in doses of 0.5 to 1.0 gram per kgm. of weight, and later Curtis and Newburgh (42) obtained mild renal injury in rats subsisting on diets containing 0.5 per cent of free cystine, and increasingly severe injury, both to kidney and liver, as the percentage of free cystine was raised above this value.

From these investigations, Newburgh is inclined to the opinion that dietary protein is one of several contributing factors in the etiology of human chronic nephritis (43).

The feeding experiments of Newburgh and his associates with rabbits may be criticized because none of the experimental diets used were demonstrably complete in other dietary factors than protein, and several of the diets were quite obviously incomplete. Hence, pathological conditions developing in experimental animals after continued subsistence upon such diets may be attributable to dietary deficiencies as well as to the excessive protein intake. The validity of this criticism is supported by the investigations of Maclean, Smith and Urquhart (44), who showed that renal injury could be readily induced with rabbits on either low- or high-protein dietaries that were obviously incomplete in other respects, and

that on such diets renal injury could be either cured or prevented by the daily consumption of cabbage leaves.[2] These experiments show that rabbits may subsist for over five months on diets containing 60 per cent of protein with no evidence of nephritis or of chronic inflammatory changes of any description. Hence, the conclusion appears justified that the nephritis observed in the experiments of Newburgh and associates, in rabbits maintained on dietaries containing much smaller percentages of protein, was probably unrelated to the protein consumed.

From a number of feeding experiments on rats it appears probable that excessive protein ingestion does not necessarily lead to kidney disease. It is true that Polvogt, McCollum, and Simmonds (45) have observed evidences of kidney involvement (hypertrophy, intense congestion, hyaline casts) in rats raised on adequate diets (largely of natural foods) containing 31 to 41 per cent of protein, but the significance of this purely morphological evidence is greatly discounted by the excellent growth and high fertility induced by the experimental diets. The infant mortality was low and, from observations extending over five generations raised upon these diets, no deterioration in nutritive condition was observed. Under these conditions, it may be doubted whether the abnormal appearance of the kidneys indicated disturbance in function.

Evans and Risley (46) have also reported the production of both glomerular and tubular damage in the kidneys of rats fed on a high-protein ration for prolonged periods.[3] However, Jackson and Riggs (47) have fed rats for as long as 20 months on synthetic diets containing 76 percent of protein (casein, or casein and egg albumin), but have been unable to produce in such animals any recognizable nephritis, although considerable hypertrophy of the kidneys (24 per cent) developed. Periodical examination of the urine showed persistent slight albuminuria in the control as well as in the high-protein rats. Drummond, Crowden, and Hill (48) and Reader and Drummond (49) have fed rats on diets containing as high as 90 per cent of protein (casein), and although adult size could not be attained on such one-sided diets, no evidence of kidney damage was at any time observed. The only effect of the abnormal excretory activity induced

[2] Possibly the high urea content of the blood and tissues in guinea pigs in which scurvy has been induced by a diet of oats only, as observed by Lewis and Karr (*J. Biol. Chem.*, 1916, xxviii, 17) was the result of a nephritic condition of the kidneys analogous to that produced in rabbits by Maclean, Smith, and Urquhart. In the former experiment, as in the latter, the addition of cabbage leaves to the diet prevented the appearance of pathological symptoms.

[3] The diets used by Evans and Risley have been characterized by Jackson and Moore (61) as being deficient in both vitamins and salt. The original article has not been accessible to the writer.

by these very high-protein diets was a marked enlargement of the kidneys. Kennedy (50) has confirmed these results of Drummond and coworkers.

Miller (51) has fed different groups of rats diets containing varying percentages of protein up to 40 per cent. These diets included varying amounts of natural foods, and vitamin, mineral and protein (casein) supplements. Although the higher protein diets induced kidney hypertrophy after 6 months of feeding, microscopic examination of the kidneys revealed no evidence of kidney damage, even in rats from which one kidney had been removed.

Addis, MacKay and MacKay (52) have raised rats from 30 days to one year of age on synthetic diets containing 70 per cent of protein (casein). These rats grew nearly to the same length as control rats raised on a normal ration, but at all ages they were lighter in weight than the controls, apparently due to an almost total suppression of fat storage in the adipose tissues. Urine examination showed a larger excretion of protein per gram of renal tissue in the high-protein rats than in the controls, though at autopsy no pathological changes were found in the kidneys. An increased weight of kidney and a decreased weight of liver were also noted in the high-protein rats. The substitution of 1 per cent of cystine for the same amount of starch in the control diet produced no observable effect on the kidneys of a third group of rats thus fed.[4]

Perhaps the most convincing demonstration of the harmlessness of protein *per se*, even when consumed in great excess, is afforded by the published investigations of Osborne and Mendel and their associates upon rats. In 1921 (53) they reported preliminary results of feeding to rats synthetic rations that consisted entirely of protein (casein) with the exception of appropriate supplies of inorganic salts and of vitamins A and B. The animals readily tripled their initial weight (about 60 grams) on such diets. Later (54) they demonstrated that rats could grow to weights exceeding 200 grams at a good rate even on these extremes of diet that furnished only minimal amounts of energy in the form of fat or carbohydrate. When the ration was less one-sided in composition, yet contained at least 50 per cent of protein, excellent growth was attained.

Having observed a striking enlargement of the kidneys in rats that were kept for some time on diets rich in protein (55), these high-protein feeding studies have been extended by Osborne, Mendel, Park and Winternitz (56) to bear specifically upon the kidney changes produced. They were able to confirm the earlier observations of large increments of body weight in rats

[4] Apparently through an over-sight, the numbers of rats used in these experiments are not reported, nor are statistical measures of variability given.

at an essentially normal rate and to adult size on diets whose protein component represented two-thirds or more of their energy content. Also animals raised to large size on diets of a relatively low protein concentration could adjust themselves with no apparent detriment to these protein-rich dietaries.

The blood of animals subsisting on these diets was somewhat richer in non-protein and urea nitrogen than the blood of control rats, but urine examinations revealed no abnormalities indicative of chronic renal disorders attributable to the protein intake. Hypertrophy of the kidneys was found to be a characteristic feature of rats subsisting on a diet in which the protein contained one-third or more of the metabolizable energy. The period of time required to bring about the hypertrophies was surprisingly brief. After 8 days of high-protein feeding, unmistakable signs of kidney enlargement were noted. Histological examinations of these enlarged kidneys showed focal lesions of varying severity and irregular occurrence, but nothing that the investigators could recognize as evidence of a nephritic condition was observed.

MacKay, MacKay and Addis (57) have shown clearly that kidney hypertrophy may be produced readily in both young and old rats by high-protein feeding (67 per cent of casein in a synthetic ration), and that there is a definite linear relationship between protein intake and renal weight applicable to rats from 70 to 400 days of age. That the hypertrophy induced in young rats was not due entirely to the added work of eliminating greatly increased amounts of urea was shown (58) by the relatively slight effect on the kidney weight of additions of urea to a diet of moderate protein content, an experimental finding in agreement with results obtained by Osborne, Mendel, Park, and Winternitz (56).

Smith, Moise and Jones (59) and Moise and Smith (60) have reported the results of experiments on rats demonstrating the much greater adaptability of the young animal to sudden increases in the excretory work of the kidney, occasioned either by unilateral nephrectomy, the ingestion of high-protein diets, or both. However, the histological evidence of renal injury induced experimentally in adult rats was not of the nature of nephritis, but resembled the senescent focal lesions that are found in aged rats. These results have been confirmed by Jackson and Moore (61), who obtained severe nephritis in only 4 out of 14 adult unilaterally nephrectomized rats fed for 10 to 14 months on a synthetic diet containing 76 per cent of casein. These investigators emphasize the importance of individual susceptibility in determining the occurrence of renal damage on the high-protein diets.

These numerous experiments on the relation between high-protein feeding and kidney structure and function have demonstrated the occurrence of extreme hypertrophy, even as high as 100 per cent, and incidentally the occurrence of extreme diuresis. That the abnormal diets used should have produced changes in the histological structure of the kidney, along with its extreme enlargement, is not surprising. The histological changes produced in the many experiments involving the feeding for many months of complete synthetic diets consisting largely of a definite protein have not been interpreted as indicative of degeneration or functional damage. The continued growth and apparent well-being of the experimental animals have confirmed this interpretation. The experimental production of definite nephritic changes in the kidney has been associated generally with the use of diets entirely or largely composed of natural foods, and consisting of only moderately high percentages of protein. In many of these cases, the diets were either obviously inadequate in other respects than protein, or quite possibly so. Granting that the renal damage has been produced by the diets used, it is not clear that the protein component is the causal factor. The latest reports of Newburgh's work (62) emphasize the differences in kidney damage secured in rats with different proteins, liver protein being most destructive, muscle protein next, and casein least. It seems fair to question whether the deleterious effects of high-liver or high-meat diets should be ascribed without further analysis to the protein content. It is clear that under certain conditions of extreme load, as in the case of unilaterally nephrectomized adult animals on high-protein rations, severe renal damage may be produced, and that with unoperated animals symptoms of renal damage may be occasionally induced by the continued ingestion of diets excessively high in protein, but it is equally clear that continued growth and well-being is possible for many months on diets containing 70 per cent or more of protein, with no evidence of functional damage to the kidney, and that this is the usual outcome.

The mass of experimental work just reviewed, demonstrating the compatibility of continued health and normal nutrition with protracted high-protein feeding, discounts considerably the significance of observations apparently relating such dietaries with vascular disturbances. Using incomplete diets and no control groups of animals, Newburgh and Squier (63) and later Newburgh and Clarkson (64) have reported the production of a diseased condition of the aorta (arteriosclerosis, atherosclerosis) in rabbits by diets rich in protein, while Nuzum, Osborne, and Sansum (65) in experiments that meet the objections raised against the former work, observed the appearance and persistence of hypertension and renal irrita-

tion in rabbits on moderately high protein rations. They hesitate, however, to ascribe these results to the protein *per se*, suggesting that diets containing excessive acid or alkaline ash might in themselves be responsible for degenerative blood vessel and kidney changes. However, Addis, MacKay and MacKay (66) have adduced evidence against such an explanation.

It may be mentioned that neither Strouse and Kelman (67) nor Mosenthal (68) have noticed any relation between protein feeding and blood pressure in observations upon patients with hypertension, while other observations of a similar nature may also be found in the older literature (69). Any adequate review of the clinical phase of the subject cannot be attempted here.

The investigations of Major and his coworkers (70) relating arterial hypertension to the production of guanidine compounds in metabolism, possibly from arginine, is interesting, but the definite demonstration of such a relation is still awaiting adequate methods for the quantitative analysis of body fluids and secretions for these compounds. The harmful effects that Hartwell (71) has noted in the young of mother rats fed upon excess protein are difficult to interpret, particularly since the rations used may not have been adequate in other respects. The "protective" effects noted on small additions of other foods is not inconsistent with this interpretation. That excess protein *per se* does not impair successful reproduction and lactation appears clearly established from the experiments of Polvogt, McCollum, and Simmonds (45), referred to above. Of the same import are the experiments of Maynard and Bender (72). Records of rats carried through from 6 to 10 successive lactations on a ration containing 50 per cent of protein failed to show any definitely unfavorable effect on reproduction or lactation as compared with rats maintained on a similar ration containing only 18 per cent of protein.

The experimental feeding of human subjects over considerable periods of time on high-protein diets has not thus far been attempted, in so far as the writer is aware. However, several reliable observations upon the Eskimos, who are essentially a carnivorous people, can serve the same purpose. A. and M. Krogh (73) in 1913 reported on the dietetic habits of the Eskimos, showing that a daily consumption of 500 grams of protein and over was not unusual and was not associated with ill-health. Lieb (74) observed that Eskimos living exclusively upon meat for long periods of time were in good health, free from constipation, and finally Thomas (75) has reported a comprehensive cardiac, vascular, and renal survey of the Eskimos of northern Labrador and of Greenland, made in 1926 by personal observation and by study of the medical records and personal experiences

of Danish physicians residing in Greenland. Quoting Thomas' report: "Nearly all of the Greenland Eskimos have no access to vegetable food and have, since being weaned, lived on an exclusively carnivorous diet, usually eaten raw. Very little fat or blubber is eaten; the red meat and liver are preferred, but with inadequate supply all parts of the animal are eaten. The observations show conclusively that there is no unusual prevalence of vascular or renal disease; scurvy and rickets did not appear among these Greenland Eskimos. The Labrador Eskimo, however, whose meat is cooked and whose diet includes many dried and canned articles, is very subject to both these deficiency diseases."

A comparison of Hindhede's extensive experiences with low-protein diets (36) with these recorded observations upon the carnivorous Eskimos justifies the conclusion that good health and successful nutrition are compatible with a wide range of protein intake.

However, the protein molecule undoubtedly contains potentialities for harm. It has been shown that intact proteins may be absorbed from the alimentary tract of animals, albeit only in minute amounts. A most interesting demonstration of this phenomenon has been reported by Mills and his associates (76) in the case of tissue fibrinogen, a powerful blood coagulant obtained from animal tissues. Its characteristic effect in quickening the clotting time of blood is noted even in very dilute concentration. In human subjects, the presence of tissue fibrinogen in blood drawn from the finger tip could be detected 2.5 minutes after swallowing a portion of this protein. Kidney excretion of the coagulant occurred in dogs after placing the substance in an intestinal loop.[5] The physiological significance of the absorption, even in minute amounts, of intact proteins rests in the peculiar reaction of the body to foreign proteins, known as anaphylaxis. The body is first sensitized to a particular protein, then responds with the symptoms of anaphylactic shock[6] to a subsequent dose, and finally on continued dosage generally becomes desensitized or immunized to that particular protein. However, with some individuals and some foods this immunization or antisensitization does not readily supervene, but rather a condition of sensitization with respect to some one or a

[5] It is interesting to note here that Mills and Necheles (*Chinese J. Physiol.*, 1928, ii, 19, 25; *Chem. Abstr.*, 1928, xxii, 1899) have noted that in general the coagulability of the blood increases after meals, an effect due to protein. Glycine also affects the clotting time of blood in the same manner as does a protein meal. This physiological effect of protein and amino acids has been related to their specific dynamic action (see also, Necheles, H., Mills, C. A., and Chu, M. K *Proc. Soc. Exp. Biol. Med.*, 1928, xxv, 451).

[6] These symptoms differ in character and severity in different animals, but involve generally a bronchoconstriction and vascular and vasomotor disturbances.

few food proteins persists, so that the ingestion of such foods results in the production of anaphylactic symptoms. This state of affairs probably accounts for many cases of so-called idiosyncrasy to a protein-containing food, such as egg white, and certain types of asthma seem to be simply an exaggerated anaphylactic response to some particular food protein to which the individual has been sensitized by previous feeding.

It is a significant fact that proteins and many of their intermediate products of hydrolysis exert profound physiological effects on animals if they gain access to the blood or the tissues. The anaphylactic reaction of proteins has just been touched upon. The so-called "proteose" or "peptone" intoxication, characterized by a marked fall in arterial blood pressure, a marked retardation of the clotting time of blood, and an increase in protein catabolism, may be referred to in this connection, while Vaughn (77) and his co-workers have shown that a very toxic body can be prepared by digesting any true protein with an alcoholic solution of sodium hydroxide. While the symptoms produced by the injection of Vaughn's "crude soluble poison" are similar in nature to those of "proteose" intoxication (78), they must be ascribed to some substance or substances other than proteoses, since they result from dosages much smaller than the effective dosage of "proteose." It is evidently an extremely important function of the intestinal mucosa to prevent the passage of these products into the portal blood or the lymph. In this connection the erepsin content of the intestinal mucosa is evidently of high significance, since protein derivatives (proteoses) do accumulate in the mucosa during digestion (79).

The relation of these protein intoxications to the amino acid make-up of the proteins has been the subject of much speculation with no unanimity of opinion. As Underhill says, "almost every study along this line points to a different amino acid as the cause of the peptone effect." There can be little doubt that the amino acid make-up of the protein bears some relation to its intoxicating effect, since gelatin, a markedly "incomplete" protein, exerts no anaphylactic effect, and on partial hydrolysis produces proteoses with no physiological action. Similarly the proteoses from zein, another "incomplete" protein, although they produce physiological effects analogous to those of typical proteoses, are much less toxic.

However, amino acids themselves are physiologically inert. The different protein intoxications or "shocks" must therefore be due, directly or indirectly, to the linkage of amino acids together in such a way that some chemical radicle, whose presence neutralizes the pharmacological action of some other radicle or larger chemical grouping, is itself neutralized. It is a significant fact that the simple decarboxylation of amino

acids, yielding amines, results in the production of substances possessing a greater or less physiological activity (80). In particular, the decarboxylation of histidine, produces an amine, histamine, possessing a powerful physiological activity and producing reactions similar in many respects to anaphylactic and peptone shock. The inactivity of histidine is apparently to be ascribed to its carboxyl group. Arai (81) has recently shown that esterification of the carboxyl group of histidine results in the production of an ester reacting similarly, though not as powerfully, as histamine. The hydrochloride of the methyl ester of histidine was found capable of causing a fall in the blood pressure of animals and of stimulating smooth musculature. However, the limiting concentration of the ester required to stimulate a piece of guinea pig uterus is 1:140,000, while that of histamine is 1:250,000,000. The ethyl esters of tyrosine, phenylalanine, cystine, and glycine were all found to be more or less active physiologically.

Proteins and their derivatives are known to consist of amino acids joined together generally in an acid-amide fashion; however, as Abderhalden has pointed out, an ester-like coupling of amino acids, involving a carboxyl and a hydroxyl group, is possible. In view of the experimental results of Arai, the thought is suggested that indifferent amino acids could become toxic as the result of the manner of their linkage together and that toxic proteins and protein derivatives may owe their toxicity to ester-like couplings in their molecules. Therefore, the fact (82) that histamine-free peptone may induce typical peptone shock in animals may not indicate any essential difference in the mechanism of peptone and histamine activity.

The actual decarboxylation of amino acids by the action of bacteria normally inhabiting the alimentary canal of animals is an established fact (83). The bacterial reactions relative to amino acids that have been investigated the most extensively are decarboxylation and deamination. According to Hanke and Koessler, the production of amines from amino acids by bacteria occurs only in acid-producing media and may be looked upon as a protective mechanism resorted to when the accumulation of H-ions within the bacterial protoplasm is incompatible with its normal life processes. The amines, according to this conception, can be thought of as reaction buffers. On the other hand, deamination results in a buffered or alkaline medium. Thus, colon bacilli in an acid medium will produce tyramine from tyrosine, while in an alkaline or a buffered medium, they will produce phenol. In an acid medium, histidine is changed to histamine, but in a buffered or alkaline medium histidine is either not catabolized, or it is catabolized with the production of products not containing the imidazole ring (84).

The action of intestinal bacteria on amino acids or partially digested proteins carried through to the large intestine (probably first hydrolyzed by bacterial proteases) include a long list of amines, fatty acids, hydroxy acids, phenols, and alcohols, that have been identified either in the intestinal contents or in the urine in which they have been excreted after absorption and detoxication of one kind or another. Particular interest has centered around the products of bacterial action of tryptophane, i.e., indole, indole ethyl amine, or less commonly, skatole, or products closely related to these, due largely to the color reactions permitting their ready determination and the study of their formation. From cystine, bacteria produce methyl mercaptan and hydrogen sulfide, and possibly also diethyl-sulfide. Ammonia is, of course, a characteristic product of putrefaction.

Many of the products thus formed are relatively harmless to the animal organism, though many of them are capable of producing profound physiological effects, as testified to by the special defense mechanism, generally supposed to be centered in the liver, against the possibility of their obtaining access to the blood. In spite of this defense mechanism, however, more or less direct and convincing evidence has been adduced connecting certain pathological conditions or symptoms with excessive putrefaction in the large intestine. Among such symptoms may be mentioned certain skin eruptions, certain types of anemia, foul breath, and even temporary sterility in women.

However, it is very easy to exaggerate the case against the harmful results of intestinal putrefaction and to magnify a mere possible or even reasonable explanation of pathological conditions into a positive diagnosis. Until recently, for example, the symptom-complex commonly designated as auto-intoxication, coincident with intestinal stasis or chronic constipation, was more or less generally, and quite reasonably, explained as the result of the absorption of histamine. However, though histamine is probably normally formed in the intestine and probably formed in increased amounts as a result of intestinal stasis, Koessler and Hanke (85) conclude that their experimental results on the absorption and detoxication of histamine may be explained fully on the assumption that it is rendered pharmacologically inert in its passage through the intestinal wall. Meakins and Harington (86) were more successful in demonstrating an absorption of histamine from different levels of the intestine, if one considers a concommittant fall of blood pressure indicative of such absorption, but they consider, nevertheless, that the balance of evidence obtained is against the view that histamine is an active agent in causing intestinal intoxication, except possibly in those cases where a definite structural deficiency, such

as ileo-cecal incompetency, is involved. Both Koessler and Hanke, and Meakins and Harington found that the liver did not exert any marked protective effect against histamine, except a purely mechanical one associated with its extensive capillary network. In the same vein, Donaldson (87) has shown that the symptoms of auto-intoxication induced by voluntary holding of feces for 100 hours, were almost immediately relieved by passage of feces in response to an enema. Such an outcome is quite incompatible with the toxin theory. According to Donaldson the typical symptoms of auto-intoxication may be primarily explained on a mechanical basis, that is, distention and irritation of the lower bowel by fecal masses, the nervous system being the distributing agency and all tissues sharing in the disquietful state.

However, the inhibition of intestinal putrefaction is considered of importance in the control of certain disorders of the intestinal tract and of certain types of dermatitis. There are conceivably two methods of inhibiting putrefaction, one involving the implantation in the intestine of aciduric types of bacteria, and the other involving diet regulation leading to a marked change in the character of the contents of the large intestine such as to favor an aciduric flora. Putrefaction in the large intestine is characterized by the production of a reaction close to neutrality (88), while carbohydrate fermentation is characterized by a reaction distinctly acid in character.

Attempts to implant aciduric bacteria in the intestine have met with varying success. If the bacterium used is a normal inhabitant of the intestine, such as *Bacillus acidophilus* or *Bacillus bifidis*, implantation may be successful (89). If it is not a normal inhabitant, such as *Bacillus bulgaricus*, implantation is unsuccessful. Even with the aciduric bacteria suitable for growth under the conditions obtaining in the large intestines, the most successful and persistent implantation involves a diet change leading to the persistence of carbohydrate residues in the large intestine.

The most practicable and effective way of modifying the intestinal flora to reduce putrefaction is to introduce into the diet certain carbohydrates so difficultly digestible or absorbable that they will persist in appreciable amounts throughout the length of the intestinal tract. Readily digestible carbohydrates, such as maltose, glucose, or sucrose, are ineffective, while lactose and dextrin are highly effective.

In conclusion, it appears that with proteins, as with other nutrients, successful nutrition and continued health and physiological efficiency are possible over a wide range of intake, though environmental and activity factors may contribute to the existence of certain optimal intakes for

certain conditions of living, but that with proteins, as with no other nutrients, the body is on continual guard against the potentially deleterious action of its intermediate digestion products, bacterial end products, and intermediate metabolites.

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